MAGNETIC RESONANCE IMAGING OF RADIATION-INDUCED THYMIC ATROPHY AS A MODEL FOR PATHOLOGIC CHANGES IN ACUTE FELINE IMMUNODEFIENCY VIRUS INFECTION

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

_____
Leah Ann Kuhnt

Certificate of Approval:

_____	_____
Nancy R. Cox Associate Professor Pathobiology	Calvin M. Johnson, Chair Professor Pathobiology
_____	_____
Frederik W. van Ginkel Assistant Professor Pathobiology	George T. Flowers Interim Dean Graduate School

MAGNETIC RESONANCE IMAGING OF RADIATION-INDUCED THYMIC ATROPHY AS A MODEL FOR PATHOLOGIC CHANGES IN ACUTE FELINE IMMUNODEFIENCY VIRUS INFECTION

Leah Ann Kuhnt

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
August 9, 2008

MAGNETIC RESONANCE IMAGING OF RADIATION-INDUCED THYMIC ATROPHY AS A MODEL FOR PATHOLOGIC CHANGES IN ACUTE FELINE IMMUNODEFICIENCY VIRUS INFECTION

Leah Ann Kuhnt

_____
Signature of Author

_____
Date of Graduation

VITA

Leah Ann (Gupton) Kuhnt, daughter of Jerry Lynn and Patricia Ann (Clifft) Gupton, was born December 30, 1973, in Paducah, Kentucky. She attended Carlisle County High School in Bardwell, Kentucky, where she graduated as Valedictorian in 1991. She graduated *cum laude*, with Honors, from the University of Kentucky, with a Bachelor of Science degree in Animal Sciences in May, 1997. She subsequently graduated *summa cum laude* from Tuskegee University, with a Doctor of Veterinary Medicine, in May, 2002. Following graduation, she entered a combined Anatomic Pathology Residency and Master of Science degree program at Auburn University's College of Veterinary Medicine. She currently resides in Auburn, AL, with her husband, Eric Sven Kuhnt, whom she married on January 5, 1993. They have one son, Andrew Eric Kuhnt, born July 25, 1994.

THESIS ABSTRACT

MAGNETIC RESONANCE IMAGING OF RADIATION-INDUCED THYMIC ATROPHY AS A MODEL FOR PATHOLOGIC CHANGES IN ACUTE FELINE IMMUNODEFICIENCY VIRUS INFECTION

Leah Ann Kuhnt

Master of Science, August 9, 2008
(D.V.M., Tuskegee University, 2002)
(B.S., University of Kentucky, 1997)

101 Typed Pages

Directed by Calvin M. Johnson

The thymus is a primary lymphoid organ responsible for the production of a diverse repertoire of immunocompetent and self-tolerant T lymphocytes (T cells), vital for proper immune function. T lymphocyte differentiation, maturation, and proliferation occur as developing cells traffic through the thymic cortex and medulla in response to a multitude of cytokines and secreted factors. The thymus undergoes physiologic involution due to aging, but also undergoes pathologic atrophy due to a variety of causes, including infectious diseases, endocrine disturbances, nutritional disorders, chemotherapeutics, or radiation injury. In particular, feline immunodeficiency virus (FIV) infection causes significant alteration and destruction of the thymus, leading to

compromise and dysfunction of the host immune system and rendering individuals susceptible to opportunistic infections, primarily through a reduction in $CD4^+$ T lymphocytes and an impairment of cell-mediated immunity. As the cat is smallest known natural model for lentiviral infection, and FIV is a significant, worldwide disease in domestic and wild cats, research involving the pathogenesis, consequences, treatment and prevention of FIV infection are of great importance. However, thymic changes often vary due to a multitude of host, pathogen, and environmental factors. A method to produce a controlled, reproducible, and highly consistent *in vivo* model of thymic atrophy was developed in this study, through the application of a single, directed dose of x-irradiation to four, 8 to12-week-old kittens. Identification, quantification, and analysis of thymic changes in irradiated and normal, age-matched subjects, were conducted using a magnetic resonance imaging protocol specifically developed to provide maximum visualization of the juvenile feline thymus. Following irradiation, marked thymic atrophy was confirmed morphometrically and histologically, and was similar to changes reported during acute FIV infection. By 7 to 14 days post-irradiation, there was a gradual rebound in thymic size, which in some instances approached pre-irradiation values. These findings demonstrate the feasibility and advantages of using a non-invasive, *in vivo* imaging technique in order to measure and evaluate changes in thymic volume, as a model for pathologic changes noted in acute FIV infection.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Calvin Johnson for his support and advice in research endeavors, as well as professional training as an anatomic pathologist. She would also like to express her appreciation to her committee members, Dr. Nancy Cox and Dr. Frederik van Ginkel, for their guidance, support, and understanding. The co-authors of the submitted manuscript, including Dr. Ryan Jennings, Dr. William R. Brawner, Jr., Dr. John T. Hathcock, and Abigail Carreno, are thanked for their contributions and hard work. Her fellow residents and pathologists have provided much educational, professional, and emotional support over the time spent at Auburn University, and are greatly appreciated. The staff and faculty of the College of Veterinary Medicine, including, but not limited to, individuals in the radiology, parasitology, necropsy, histopathology, clinical pathology, animal care facilities, and various research laboratories have been a wonderful and invaluable support. Special thanks are extended to Dr. Fred Hoerr, for providing the encouragement and opportunity necessary to begin this undertaking. Finally, and most importantly, the author expresses her utmost gratitude for the limitless support, love, and encouragement received from her family.

Style manual or journal used: Veterinary Radiology and Ultrasound

Computer software used: Microsoft Word, eFilm Lite, ImageJ, Endnote, GB-Stat

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. LITERATURE REVIEW

The Thymus

The thymus, located in the cranial mediastinum and caudal cervical region, is derived embryologically from ectoderm of the third branchial cleft and endoderm of the third pharyngeal pouch, and is composed predominantly of lymphoid, epithelial and mesenchymal components.[171] It is a primary lymphoid organ, populated by lymphoid precursors emigrating from sites of hematopoiesis, such as the bone marrow in the adult, or the fetal liver and spleen.[9] The thymus is responsible for the development of immunocompetent T lymphocytes (T cells) from precursor cells arriving from the bone marrow, the proliferation of clones of mature naïve T cells, and the development of immunological self-tolerance. In fact, T cells derive their name from the fact that they arise in the thymus. Arranged in lobes and lobules, with minor species variation in lobar architecture, and surrounded by a connective tissue capsule, the thymus histologically appears to be composed of densely cellular outer cortex and a relatively sparsely cellular inner region or medulla (Figure 1). In addition to a large circulating and continually migrating lymphoid cell population, this lobulated organ also consists of a significant epithelial and mesenchymal stromal framework.[10, 85] The mesenchymal stroma consists primarily of macrophages, Langerhans-like dendritic cells, and perivascular reticular connective tissues. Development and maturation of T-cells is under the influence of the thymic epithelial and mesenchymal stroma, as this framework provides support through

physical and direct cellular interactions, and also has immunomodulatory and neuroendocrine functions, producing cytokines and other soluble factors, which act in autocrine, paracrine, or hormonal capacities.[24, 210] Many factors, such as thymic stromal lymphopoietin (TSLP),[110, 120, 261] keratinocyte growth factor (KGF),[64, 70, 196] and IL-7,[79, 198, 209] are necessary for normal homeostasis and thymopoiesis, as well as the orderly maturation of functional T lymphocytes. The thymus is thus responsible for the production of a diverse repertoire of immunocompetent and self tolerant T lymphocytes, vital for proper, sufficient, and regulated immune function.[61, 111] The thymus is physiologically most active during the fetal and early postnatal period. At the time of sexual maturity, it begins to undergo significant atrophy, with reduction in size and infiltration or replacement by adipose tissue, as part of normal physiologic involution. It does not become completely dormant at this stage, however, but continues to provide immune support through production of naïve T cells, playing a vital role in modulating both cell-mediated and humoral immunity.[91] Thymic size and function is also affected by a multitude of factors, including stress, malnutrition, endocrine disorders, neoplastic processes, radiation, chemotherapeutics, and a multitude of infectious diseases. Atrophy, both physiologic and pathologic, is characterized predominantly by structural and morphological alterations, increased levels of thymocyte apoptosis, and overall hypocellularity. Strategies aimed at repopulation of the atrophic thymus, with augmentation or support to enhance function, is currently a field in which there is abundant active research.

Thymocytes, the lymphocyte precursor cells within the thymus, are uniquely and highly susceptible to productive immunodeficiency virus infection, which is particularly

devastating in neonatal or juvenile patients, who rely on thymic output to maintain T lymphocyte homeostasis.[95, 253] Decreased thymic size and impaired function are major contributors to the rapid and severe course of disease noted in pediatric and neonatal disease, as those infected at an early age experience much shorter incubation times, accelerated disease progression, and a higher frequency of clinical symptoms, as compared to those infected as adults.[83, 176] Upkeep of the normal thymic microenvironment is therefore crucial to maintaining adequate T lymphocyte production and functional output. Disturbances of this network, as occurs in various diseases such as infection with FIV,[113, 169] classical swine fever (CSF) virus,[206] *Trypanosoma cruzi*,[133, 145] or some bacteria,[218] can have dire consequences on T lymphocyte numbers. However, the thymus is capable of partial regeneration and restoration of functional capacity following supportive and anti-retroviral therapy.[6, 96, 190, 242] Innovative new treatment modalities, including stem cell therapy and gene therapy, may soon augment more traditional forms of antiviral therapy. In order to better understand the changes that occur in the thymic microenvironment, an animal model that mimics the physical, immunological, and biochemical parameters occuring during times of atrophy and regeneration, would be exceedingly useful.

Thymic Involution and Atrophy

The thymus undergoes a normal process of physiologic involution with age, in which there is a uniform reduction in thymic size and decreased overall cellularity. Thymic involution is defined as a normal, gradual, age-associated change in thymic cellularity, while the term atrophy is usually applied to pathologic changes induced by causes other than aging.[186] In older animals, there is usually a small thymic remnant, and

involution with complete loss of the thymus typically does not occur in any species.[186] The most visible gross physical and histologic changes within the involuted thymus include a marked reduction in size and an infiltration or replacement by adipose tissue. Histologic changes include alteration of normal architecture, enlarged perivascular spaces, leading to decreased thymic epithelial space, reduced cortical thickness, and loss of corticomedullary demarcation.[98, 225] There is diffuse hypocellularity, with overall reduction in lymphocytic cell numbers, particularly within the cortex, with relative sparing of the medulla. There may be increased numbers of perivascular B cells and plasma cells, particularly at the corticomedullary junction, and formation of lymphoid follicles with germinal centers.[186]

Thymic involution is closely associated with the onset of puberty and sexual maturity, suggesting that involution is partially dependent of increased levels of circulating adrenal and sex hormones.[90] Sex hormones continually play significant roles in the maintenance and regulation of the thymus. Gonadectomy of both young male and female rodents will delay involution, while castration of older animals will result in thymic enlargement and hyperplasia, with increased numbers of thymic and splenic T and B cells.[122, 236] Testosterone, estrogen and hydrocortisone treatments have resulted in marked thymic involution, while only mild to moderate changes occurred with progesterone administration.[25] Transient involution of the maternal thymus also occurs during pregnancy, in which increased levels of estrogens inhibit thymocyte development and T cell production.[200, 201]

The roles of various other hormones on thymic involution have also been extensively studied, including those of thyroid and growth hormones. Loss of thymic

tissue with aging is associated with reduced levels of circulating growth hormone (GH).[130] Histologic evaluation of aged rats treated with GH had morphologic evidence of thymic regeneration, as well as reconstitution of hematopoietic cells in the bone marrow.[78] Growth hormone can enhance thymic function, with increased numbers circulating naïve and total $CD4^+$ T cells.[159] GH, mediated by insulin-like growth factor-1 (IGF-1), increases thymic epithelial cell (TEC) proliferation *in vitro* and influences *in vivo* thymocyte traffic within the lymphoepithelial complexes, the thymic nurse cells, and modulates the homing of recent thymic emigrants.[213]

Administration of thyroxin in low doses resulted in thymic hypertrophy, while high doses caused thymic atrophy, indicating a dose-dependent response.[25] Interestingly, thymic hyperplasia is also noted in Grave's disease, a type of autoimmune hyperthyroidism, in which there may be massive and radiologically detectable enlargement of the thymus.[32, 86] It is thought that the thymic hyperplasia is a result of, not a cause of, the autoimmune and hyperplastic changes within the thyroid, as thyroid changes persist even after thymectomy.[238] Alternatively, thymic hyperplasia has been noted to disappear following resolution of hyperthyroidism and return to a euthyroid state.[259] The exact underlying mechanism by which this occurs has not been determined, but may deal with increased levels of thymulin[65] and other thymic hormones, as modulated by secretion of thyroid hormones, especially triiodothyronine (T3).[66]

Seasonal changes to the thymus are noted in animals that hibernate during winter months, in which the thymus undergoes annual atrophy and involution, with substitution by abundant, energy-rich multilocular adipose tissue, also known as brown fat.[125] At the end of hibernation, there is reduction of thymic brown fat, with repopulation by epithelial

and stromal tissues, as well as increased infiltration by lymphocytes. It is proposed that changes in cellular TNF release, along with the changes in the levels of multiple hormones, such as melatonin and corticosteroids, play a significant role in the rise and fall of thymic activity due to hibernation.[170]

Loss of thymic function and size in aging is thought to result from loss of support from the microenvironment stroma and associated cytokines, as well as intrathymic and extrathymic hormonal influences. Thymic involution and decreased proliferation of T lymphocytes can be partially restored by thymic tissue transplantation or administration of intrathymic hormones.[27] Thymic function is at its most active during the fetal and perinatal periods, a time when there is exposure to a multitude of novel environmental and foreign antigens. Although the thymus is necessary for the production of new naïve T cells in the neonate and juvenile, it has been demonstrated that the adult thymus is also capable of T cell reconstitution, albeit at a reduced capacity.[11, 98, 108] Aging is associated with a reduction in the contribution of the thymus to the naïve T-cell pool, but with no significant decline in the total number of T cells in the peripheral T-cell pool.[11, 103, 235]

Many non-infectious and infectious disease states are accompanied by thymic changes (Figure 2). Stress and excess endogenous corticosteroid release can cause thymic atrophy, characterized by decreased cellularity, decreased cell density, and decreased functional compartment size, secondary to apoptosis.[61] The thymus is the most sensitive lymphoid tissue to changes in adrenocortical hormone levels, but these changes may be reversible.[186] This differs from age-related involution, in which there is a gradual loss of supportive structures, so that acute reductions in thymic size due to stress are more easily corrected with removal of the stressor.[92] Although exact mechanisms are unknown,

apoptosis due to oxidative stress may play a large role in glucocorticoid-induced apoptosis.[140] It has also been hypothesized that intracellular alterations in ATP and Ca^{2+} levels may be responsible for death of thymocytes.[77] In particular, the $CD4^{+}CD8^{+}$ double positive thymocytes seem to be particularly sensitive to increases in extracellular ATP, which results in Ca^{2+}-dependent membrane hyperpolarization, cytosolic acidification, and DNA fragmentation.[157]Additionally, stress may be incited by or accompanied by changes in nutritional status, behavior, or concurrent disease, etc., causing a multifaceted disruption of thymic and immune system homeostasis.

During numerous bacterial, viral, protozoal, and parasitic infections, there may be variable thymic atrophy. This may be due to direct cellular infection with lysis or apoptosis of the affected cells, or an indirect response to a multitude of interrelated hormonal, nutritional, and microenvironmental factors. Disruption of the tissue and organ homeostasis leads to changes deleterious to lymphocyte production. The degree of thymic involution is often correlated with the duration or severity of illness.[145, 237] $CD4^{+}CD8^{+}$ double positive cells are the most sensitive cell population and more often affected than other subpopulations, leading to decreased numbers of $CD4^{+}CD8^{+}$ cells within the thymus.[145] Changes within the thymus of diseased animals, such as those infected with *Trypanosoma cruzi* in Chagas' disease, do not appear to be associated with stress or glucocorticoid release, since adrenalectomized mice have similar patterns of thymus atrophy.[133] Other diseases, particularly lentiviral infections such as HIV and FIV (discussed in detail later), have significant alterations in thymic size, appearance, and function.

Lymphoid Population of the Thymus

In order to better evaluate and augment the ability of the thymus to regenerate following times of physiologic or disease-induced atrophy, normal patterns of thymic lymphoid emigration and population must be understood. It is known that lymphoid precursors cells arrive from sites of hematopoiesis, ultimately originating from a continually renewing population of hematopoietic stem cells (HSCs).[216] These pluripotential stem cells have the capacity to differentiate into multiple different lineages, including myeloid, monocyte/macrophage, dendritic, erythroid, and lymphoid precursor cells. Secreted cytokines aid in the differentiation and commitment of these precursor cells. For example, granulocyte colony-stimulating factor (G-SCF) induces initiation and production of granulocytes.[234] Even in adult life, in which there is physiologic involution, there is evidence that the mature thymus is maintained by hematogenous precursors.[74]

The thymus does not have a population of self-renewing hematopoietic precursors, and these must therefore be imported and replenished from sites of hematopoiesis, such as the bone marrow.[97, 203] There is question as to whether this process is continuous or intermittent. Studies using chimeric mice have shown that the thymus is dependent upon blood-borne prothymocytes, and intrathymic precursors are replaced at a rate of 2 to 3% per day in the adult.[57] However, it was not clear if the replacement occurred as a continuous action or in discrete waves.[74] In more recent kinetics studies, these precursor cells were shown to periodically infiltrate niches within the thymus through a gated phenomenon, in which a receptive period of approximately one week was followed by a refractory period of approximately three weeks duration.[74, 75] These waves are discrete, but may be overlapping.[56] This provides support the theory that

there might be a type of feedback loop regulating homing to and population of the thymus. This would involve multiple chemotactic and adhesion factors, acting with microenvironmental niches, to stimulate and enhance thymic population and thymocyte differentiation.[75] It is also thought that this occurs in concert with signals to the bone marrow, coordinating release of precursor cells.[56]

As occurs in most organs, the progenitor cells which leave the bone marrow and emigrate to the thymus are recruited through multistep adhesion cascades and through the action of homing molecules and associated receptors.[216] It is thought that these precursor cells enter the thymus at the corticomedullary junction,[138] then migrate outward through the cortex, and return inward again during development and maturation into self-tolerant, major histocompatibility complex (MHC) -restricted, and immunocompetent T cells.[112] To date, it has been difficult to determine the earliest population of precursor cells which enter the thymus from the hematopoietic sites, but there are several candidates. These include the HSCs, early thymic progenitors (ETPs), and more differentiated common lymphoid progenitors (CLPs).[109, 216] There is some debate as to whether the cells entering the thymus are lineage-committed at the time of entry. The Notch signaling pathways, broadly involved in cell-fate decisions and differentiation processes, are known to play a significant role in the commitment of progenitor cells to that of T cells, but the exact mechanism is unclear.[109] Notch1 is essential in determining B vs. T cell development, as loss of Notch1 function in mice had a marked deficiency in thymocyte development.[194] The earliest population of cells arriving in the thymus are considered to be triple negative, in that they are functionally negative for CD3, CD4 and CD8 markers.[254]

Entry of thymic precursors from the bloodstream requires migration from the vascular lumen and entry into the thymic parenchyma at an appropriate location, and a key feature of HSCs is their ability to migrate in a site-specific fashion.[121] This is a highly regulated process, involving molecules and receptors on both the endothelial cells lining the vessel wall, underlying stromal cells, and the circulating progenitor cells. These recruitment and selection processes are similar to that which occurs in inflammation, which requires mobilization of leukocytes. Selectins are transmembrane adhesion molecules on endothelial cells (E-selectin and P-selectin), platelets (P-selectin), and leukocytes (L-selectin) that bind to carbohydrate ligands on target cells, causing slowing and rolling, so that migration can occur.[229] The next step involves more avid binding of the migrating cell to the endothelial surface via integrins. Integrins are heterodimers primarily found on leukocytes, that function in cell attachment to the extracellular matrix and signal transduction.[222] Homing of progenitor cells to the thymus depends on P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) interactions,[127] as it participates in the first step of adhesion and entry into the thymic postcapillary venules.[216] Supportive of this notion is that PSGL-1-deficient mice had decreased numbers of some ETPs. Following selectin binding, a $G\alpha_i$-protein coupled signal results in the activation of integrins, mediating firm adhesion to the vascular wall, through interactions such as $\alpha4\beta1$ with vascular cell adhesion molecule-1 (VCAM-1).[216] Although early progenitor hematopoietic stem cells enter the thymus using a homing receptor, this process also requires thymotaxin, a peptide secreted by the reticulo-epithelial (RE) cell network.[26]

T Cell Production

Lymphocyte precursor cells arise in hematopoietic organs from pluripotential stem cells. This predominantly occurs in the yolk sac during embryonic development, and later the fetal liver and spleen. In most species, the bone marrow becomes the primary hematopoietic organ around the time of birth.[9] As in the thymus, the bone marrow has an essential network of stromal cells, which influences cellular development along either lymphoid or myeloid pathways. Cytokines and secreted factors, which can act in hormonal, autocrine, or paracrine fashions, are necessary for the development and maturation of T cells in the thymus.

The most immature thymocytes express neither the T cell receptor (TCR) complex in association with CD3 nor the accessory molecules CD4 or CD8 and are referred to as being $CD4^-CD8^-$ double negative (DN) or $CD4^-CD8^-CD3^-$ triple negative (TN). These DN cells make up approximately 5% of the thymic lymphocyte population.[212] Many additional cell surface markers are used to characterize early and developing thymocytes, including CD25 and CD44.[87] Utilizing these markers, DN cells with $CD25^-CD44^{high}$ are referred to as DN1 cells. After entering the thymus from the bloodstream near the corticomedullary junction, these DN progenitor cells migrate outward towards the capsule.[138] This involves cell-to-cell interactions with VCAM-1 positive stromal cells,[188] as well as interactions with extracellular matrix compounds, including laminin, fibronectin, vitronectin and collagen.[211] As they migrate, they proceed through additional stages of maturation, namely DN2, characterized as $CD25^+CD44^{high}$ and DN3, which are $CD25^+CD44^{low}$.[151] These $CD25^+$ subsets are the most numerous of the DN subsets.[87] Differentiation to the DN4 $CD25^-CD44^-$ subset occurs in the cortical

subcapsular zone.[188] It is also here that the cells become $CD4^+CD8^+$ double positive (DP), coinciding with a reverse in polarity and migration back towards the inner cortex. DP cells make up approximately 80-90% of the thymic lymphocyte population.[212]

Immature DN thymocytes express a pre-TCR complex, consisting of CD3 and a heterodimer of the TCR β-chain and a pre-Tα chain.[205] Once a signal is transmitted through the pre-TCR, this induces the DP $CD4^+CD8^+$ expression and halts β–chain gene rearrangement. The DP thymocytes then proliferate. Thymocytes that do not achieve a productive TCR gene rearrangement die by apoptosis.[212]

Double positive thymocytes, as they move into the deep cortex, undergo positive selection, which involves interaction of the thymocytes with MHC molecules, which are complexed with endogenous antigens on cortical stromal epithelial cells. This allows for the selection of only the thymocytes whose receptors exhibit self-MHC restriction. Only those thymocytes which bind the MHC complex receive a positive signal for survival.[150] If the interaction is with MHC class II or class I, cells differentiate to become either $CD4^+$ or $CD8^+$ single positive (SP), respectively. Studies utilizing MHC class I- or II-deficient mice resulted in failure of production of $CD8^+$ thymocytes and $CD4^+$ thymocytes, respectively, supporting the importance of MHC molecule binding in positive selection.[45, 239]

Following positive selection, only single positive cells enter the medulla, where maturation continues and cells become functional. It is thought that chemokine responsiveness is upregulated after positive selection, which prevents less mature double positive cells from entering the medulla.[188] It is here in the medulla that negative selection occurs, which is the process of eliminating cells whose antigen receptors avidly

bind endogenous antigens are selected against and undergo apoptosis. These endogenous antigens are complexed with MHC on antigen presenting cells such as dendritic cells and macrophages. This prevents reactivity to self-antigens and results in central tolerance. The overwhelming majority of thymocytes produced undergo apoptosis, in part due to the effects of positive and negative selection. Those that survive may be released to the circulation as mature, naïve, single positive T cells.

Thymic Cytokines

Cytokines are small secreted proteins, produced *de novo* in response to an immune stimulus, which mediate and regulate immunity, inflammation, and hematopoiesis.[88] They generally act over short distances and short time spans and at very low concentration. Cytokines are redundant in their activity, meaning similar functions can be stimulated by different cytokines. Cytokines are often produced in a cascade, as one cytokine stimulates its target cells to make additional cytokines, and they can also act synergistically.[160]

There are functionally distinct types of $CD4^+$ T helper cells, including Th1, Th2, and Th17 subsets, distinguished by the cytokines that they produce and the manner by which they respond to immune stimulation.[248] These subsets of T cells secrete cytokines which promote different types of immunologic responses, including stimulation of cell-mediated immunity and inflammation, and stimulation of B cells to produce antibody. In any type of immune response, there exists a mixture of these activities, but this may be skewed or imbalanced, so that one cytokine response pathway predominates. T cells are initially naïve Th0 cells, but become polarized to responses such as Th1 or Th2 based on the nature of the offending antigen or pathogen and the resultant cellular and cytokine

environment present.[123] Th1 cells produce IL-2, IFN-γ, and TNF-β, which activate cytotoxic T cells and macrophages, stimulating cellular immunity and inflammation. Th1-type cytokines tend to produce the pro-inflammatory responses needed for the destruction of intracellular parasites, and they also assist in antitumoral immunity, hypersensitivity reactions, and perpetuate autoimmune responses.[21, 123] Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 for the mediation of antibody responses.[148] Type 2 polarized T cells are effective at controlling extracellular pathogens, such as helminths, or assisting in the promotion of humoral immunity.[71, 89] Th17 cells are a relatively newly recognized subset of T helper cells, characterized by the production of IL-17, important in the regulation of autoimmunity and aiding Th1 and Th2 responses in intracellular and helminthic infections, respectively.[93, 246] IL-4 stimulates Th2 activity and suppresses Th1 activity, while IL-12 promotes Th1 activity. IFN-γ inhibits the development of Th2 cells, but IL-10 inhibits Th1 secretion of IFN.[172, 173] Th17 cells differentiate in response to IL-23, in the absence of IL-4 and IFN-γ. Thus there is much antagonism and a complex relationship between the activities of these major classes of cytokines.

Thymic epithelial cells (TECs) and thymocytes are the main source of cytokines within the thymus, although all cells participate in their production to some degree.[248, 260] As thymocytes mature from DN1 to DP stages, the ability to produce cytokines and express cytokine receptors is gradually reduced, reflecting decreases in the cytokine dependence of the respective processes as they proceed through maturation. After the completion of the selection process, however, the capacity of thymocytes to produce cytokines and respond to them is restored.[260] The cytokine environment of the thymus varies according to location, predominant cell types, and responses generated by outside

influences.[189] With atrophy, hyperplasia, or disease, these microenvironments can alter dramatically. However, in contrast to peripheral T cell populations, thymic T cells and their cytokines are not often involved in inflammation, but more so in the roles of guiding thymocyte proliferation, differentiation, and maturation.[202]

HIV infection induces changes in cytokine production, which may be significant in the progression of clinical disease, impacting viral replication and immune function. What factors or mechanisms that allow the development of symptomatic disease, however, are not explicitly known, but a general trend from Th1 to Th2 T-helper cell responses has been implicated.[156] A strong cellular immune response (Th1) appears to be of importance in maintaining nonclinical disease. During FIV infection, thymic levels of IFN-γ, IL-12, and IL-10 increased, likely in association with inflammation, while control animals increased thymic IL-4 and IL-12.[53] Altered cytokine levels in control animals may have been the result of physiologic changes due to growth and/or involution. In multiple examined lymphoid organs, there was a trend towards elevation of Th2 cytokine levels, but this was not strictly adhered to, as IL-10 levels were also often increased. In a separate study, IFN-γ levels were increased 10-fold in thymocytes of FIV infected cats versus sham-inoculated controls.[181]

Selected Cytokines

Several strategies have been proposed in an effort to combat the decline of T cell numbers. Certain cytokines have shown promise in the ability to stimulate and restore the thymic lymphopoietic potential. The most studied and investigated to date is IL-7, but other cytokines and cellular factors have therapeutic and regenerative potential, including keratinocyte growth factor (KGF), insulin-like growth factor 1 (IGF-1), and IL-15. It is

not straightforward, however, as the interconnectivity and interrelated nature of short-lived cytokines and paracrine functions.

IL-7, originally referred to as pre-B cell growth factor or lymphopoietin 1,[39] is a 25kDa protein constitutively produced by thymic stromal epithelial and mesenchymal cells, bone marrow stromal cells, dendritic follicular cells, dendritic cells, keratinocytes, hepatocytes, and intestinal epithelial cells, and acts prominently in T and B cell development.[13, 20, 209] There is considerable homology between IL-7 genes of various species, with 80% homology between mouse and human sequences.[20] The IL-7 receptor is composed of an IL-7Ra chain and a common γ-chain cytokine receptor, which is also seen in the IL-2, IL-4, IL-9, and IL-15 receptors.[7, 214] Signal transduction involves the Jak/Stat pathway,[76] which can lead to proliferative, anti-apoptotic, and activation signals.[20] IL-7 is known to be essential for the survival and differentiation of thymocytes, possibly as a cofactor for VDJ rearrangement.[101] In IL-7 knockout mice, there is significant reduction in pro-T cell numbers and decreased γδ-TCR thymocytes.[241]

IL-7 expression is up-regulated in a number of lymphopenic conditions including those that cause depletion or suppression of the marrow, as occurs following chemotherapy or HIV infection.[209] Plasma IL-7 levels inversely correlate with $CD4^+$ T-cell counts in many of these conditions, as IL-7 levels are increased in HIV infection along with lymphopenia.[6] IL-7 levels are also increased 2.5-4 fold in mice following dexamethasone induced thymic atrophy,[262] but these changes were transient and levels returned to normal levels by 14 days post-treatment. Additionally, estradiol- and gamma radiation- induced atrophy have produced prolonged increases in IL-7 levels. IL-7 mRNA production is radiosensitive and is inversely proportional to the radiation dose.[40] This has

important implications in treatment of patients via bone marrow transplantation. Other factors, including c-kit ligand and some integrins are also deficient following radiation.

There has been much interest in therapy with IL-7, either through gene therapy or direct IL-7 delivery. Exogenous IL-7 increased thymocyte proliferation and TREC levels in thymic organ culture and in thymic grafts to NOD-SCID-hu mice.[180] Direct injection of IL-7 –secreting thymic stromal cells, transfected with a constitutive IL-7 expressing plasmid, resulted in sustained improvement in early thymopoiesis, with augmentation of bcl-2 expression.[190] Even more convincingly, therapy of SIV-infected nonhuman primates with recombinant human IL-7 resulted in significant increases in peripheral blood $CD4^+$ and $CD8^+$ T cell numbers.[80] However, as with any therapy, care should be exercised, as overexpression of IL-7 in transgenic animals results in excessive lymphoid proliferation and lymphoma.[72, 73, 197]

Thymic stromal-derived lymphopoietin-1 (TSLP-1) is a cytokine produced by thymic stromal cells with a structure and function similar to that of IL-7.[20] This homology may partly contribute to the slight production and maturation of B and T cells found in IL-7 knockout mice. TGF-β can suppress IL-7 production by bone marrow stromal cells, and IL-7 conversely inhibits TGF-β production by fibroblasts and macrophages, in a negative feedback mechanism.[102]

IL-15 is produced by monocytes/macrophages, dendritic cells, bone marrow stromal cells, thymic epithelial cells and multiple other tissues, including placenta, skeletal muscle, kidney, lung, heart, and epithelial cells.[68, 69] IL-15 stimulates T cell proliferation and is necessary for the development and activation of γδ T cells and NK cells, as well as for induction and maintenance of $CD8^+$ memory cells. It shares the γ

common chain also found in IL-2, IL-4, IL-7, and IL-9. IL-15 function is similar, yet distinct from that of IL-2,[50] and involves activation of the JAK/Stat pathway, induction of Bcl-2, and stimulation of the Ras/Raf/MAPK pathway.[31] It has been proposed as an adjuvant for vaccines, as an immunomodulator to enhance activity against intracellular pathogens.[129] Recombinant feline IL-15 has been shown to react with a commercially available human IL-15 ELISA kit.[50] IL-15 stimulates proliferation of memory $CD4^+$ and $CD8^+$ cells and naïve $CD8^+$ cells,[119] and is considered to be a pro-inflammatory cytokine, inducing chemotaxis of T cells.[250]

Keratinocyte growth factor (KGF) is a 28-kDa member of the fibroblast growth factor (FGF) family of molecules and a potent epithelial cell mitogen.[5] It is produced by thymic mesenchymal cells and by T cells at various developmental stages.[204] KGF production is upregulated following epithelial cell injury, and its actions in damaged intestinal epithelium included the stimulation of cell proliferation, migration, differentiation, survival, DNA repair, and detoxification of reactive oxygen species.[70] A receptor isoform of KGF (FgfR2IIIb), is expressed exclusively in the thymus on cortical and medullary epithelial cells.[204] Mice deficient in FgfR2-IIIb show a block in thymic growth.[196] A recent study of mice injected with KGF resulted in increased numbers of thymocytes, initially due to proliferation of immature triple negative cells, but continued with increases in more mature phenotypes as well.[204] Enhanced T cell export correlated with an increase in thymic size and increased absolute thymic cell numbers.

IL-12, a heterodimeric cytokine composed of 40 kDa and 35 kDa subunits, stimulates CTL activity, enhances NK activity, and induces IFN-γ production,[104] and is thus essential for Th1 responses. IL-12 knockout mice have increased rates of thymic

involution in aged but not young mice, and is associated with histologic degeneration of the thymic extracellular matrix and vascular network, and extensive changes in architecture and organization.[136] Additionally, there was increased apoptosis of cortical and medullary thymocytes, as determined via terminal dUTP nick-end labeling (TUNEL). This suggests IL-12 plays a major role in T cell survival. IL-12 enhances the proliferative response induced by IL-7 or IL-2,[136] while stimulating T cell production of IL- 2 and IFN-γ.[156] Increases in IFN-γ production have been noted in FIV infections to coincide with a decrease in FIV RT activity, following the addition of IL-12 to cell cultures.[156]

IL-10, produced by a variety of cell types, including B cells, macrophages, and keratinocytes, can induce Th2 humoral responses, along with IL-4 and IL-6 production, and may be associated with clinical disease.[156] IFN-γ and IL-10 were upregulated in the thymus, as well as in the peripheral lymph nodes of acutely and chronically FIV-infected cats.[137] IFN-γ was produced by both CD4 and CD8 thymocytes, while IL-10 was produced primarily by CD4 thymocytes. Expression of IL-2, IL-4, IL-12, and TNF-α was decreased during acute infection. Acutely FIV infected cats have been reported to constitutively express high levels of IFN-γ, TNF-α, and IL-10 in the peripheral lymphoid tissue.[51]

TNF-α levels in HIV patients have yielded conflicting results, but it has been demonstrated that increased TNF-α levels correlated with the progression of clinical disease.[141] Serum levels of IL-6 have also been reported to increase with HIV infection. Alveolar macrophages from symptomatic and asymptomatic HIV patients produce more

TNF-α, IL-1β and IL-6β. However, in a study of FIV-infected cats, there was a decrease in TNF-α production and no change in IL-6 production in stimulated macrophages.[141]

Insulin-like growth factor I (IGF-I), along with growth hormone (GH), plays a critical role in the growth and development of the thymus, as it does in multiple other organ systems, particularly in early postnatal and prepubertal stages. IGF-I may act by increasing the migration and colonization of bone marrow-derived precursors to the thymus.[155] Cats affected with GM1 gangliosidosis, which have neurological dysfunction, stunted growth, and abrupt, premature thymic involution have been shown to have significantly decreased levels of serum IGF-1.[44]

Magnetic Resonance Imaging of the Thymus

Different modalities of thymic imaging vary in their capacity to differentiate the thymic parenchyma from surrounding soft tissues.[29] Magnetic resonance imaging (MRI) of the hilum, pericardium, mediastinum, and its contents provide substantial improvement in terms of tissue contrast, differentiation and resolution, as compared to conventional imaging modalities, particularly when viewing soft tissue densities. MRI is a sensitive method for identifying closely associated fat and water in microscopic mixtures.[227] Additionally, MRI provides superior detail of thymic margins,[223] which is particularly useful for measurement studies as conducted in this project.

Other methods, such as fluorodeoxyglucose (FDG)-positron emission tomography (PET) scanning, which provides information regarding tissue glucose metabolism, have been utilized in various thymic imaging studies. PET scans have been used extensively in mouse thymic studies, but often in conjunction with anatomic images from a MR or CT unit.[99] PET scanning alone is less reliable in discriminating normal thymus from that of

areas of thymic hyperplasia or neoplasia.[252] Increased FDG uptake is seen not only in cases of neoplasia but also in normal organs, referred to as physiological FDG uptake, and may cause misinterpretation of results.[158] However, there are obvious merits to many various imaging modalities, and these are not to be discounted. For example, CT is the modality of choice for thymomas,[29] and PET scans are particularly useful in detecting tumor spread and metastasis.[29, 226]

Magnetic resonance imaging provides superior detail and contrast of soft tissues throughout the body. These nuclear magnetic resonance signals are created by radiofrequency (RF) energy excitation in the presence of a magnetic field.[207] MRI differs from other imaging modalities in that the types of physical and chemical bonds and amounts of chemical elements present, their thermal motions, and the chemical interactions strongly affect the signal that is given off.[22] MRI is also a relatively rapid mode of imaging, is capable of relatively high resolution, variable orientation of scan planes, and generation of three-dimensional images. In MRI studies, another clear advantage is the ability to provide longitudinal data from the same subject over time.

MRI uses manipulation of magnetic fields, an additional advantage over CT, which uses ionizing radiation. The alignments of nuclear magnetized fields in MRI can be altered to produce a rotating magnetic field detectable by a scanner. In clinical usage, imaging primarily involves hydrogen atoms in free water, which are inherently susceptible to a magnetic field.[207] In the absence of an external magnetic field, protons spin in random directions, but become aligned when the magnetic field is present. While in the presence of the strong primary magnetic field, the organ or tissue to be scanned is simultaneously subjected to a second magnetic field, which oscillates at different

radiofrequencies and in planes perpendicular to that of the main field, pushing some protons within the main field out of alignment and causing excitation.[28] The resulting MR images represent the contrast between different kinds of tissue and the pathologic alterations within them, based on the differences in relaxation times of the tissue, in which protons realign.[28] The timing between the RF pulses is the repetition time (TR), while the timing from the pulse until the signal is acquired in the receiving coil is the echo time (TE).[185] The recovery process along the longitudinal plane of the RF pulse is the spin-lattice relaxation (T1), while along the transverse plane it is known as the spin-spin relaxation (T2).[185] Thus different tissues, with different decay and recovery times, result in contrasting images. The signal intensity is dependent on the proton density in the tissues, the intermolecular interactions for a given proton defined by the T1 and T2 relaxation times, and the bulk flow of the protons. MRI has an enormous variety of pulse sequences that may be utilized to give information on such topics as morphology, motion, flow, diffusion, function, etc., leading to abundant versatility in its uses.[28]

Within the thymus, because of the common mixture of soft tissue elements, MRI is particularly useful. Water has both a long T1 and T2, while fat has a relatively short T1 and T2.[207] This enables visualization and differentiation of the different tissue types, which vary with respect to water and fat content. Additionally, the thymus is often apposed with lung tissue, which is inherently proton poor, due to filling with air, and provides marked image contrast. Fast spin echo has the advantage of speed of the sequences, as well as their low sensitivity to magnetic susceptibility artifacts and magnetic field heterogeneities.[100] Fast spin echo can also be combined with other techniques, such as the short tau (t, or inversion time, TI) inversion recovery (STIR)

technique, to enhance visualization. STIR is considered a fat suppression technique, as it utilizes an inversion recovery pulse sequence with specific timing so as to suppress the signal from fat. An inversion recovery pulse sequence is a spin echo pulse sequence preceded by a 180° RF pulse.[28] In the standard STIR sequence, the spin echo sequence is thus completed by a previous 180° inversion pulse. This takes advantage of the naturally short T1 of fat, when imaging protocols use a short TI, such as 135 ms, as used in the feline studies presented here. The combination of STIR and fast spin echo sequences reduces acquisition time, while offering fat signal suppression techniques with low sensitivity to magnetic field heterogeneities.

Not only is there variation among species in the appearance of the thymus grossly and in imaging studies, but there is often marked variability of the thymus between individuals of the same species.[149] Further variability is induced by age, nutritional status, stress, and concurrent disease. There may be alterations in thymic size, shape, density, and composition. Motion within the chest cavity, due to respiration and the beating of the heart, may induce artifactual changes or momentarily alter outlines. Hyperplastic or neoplastic changes may result in minimal to mild thymic enlargement and may be difficult to assess. Changes in pliability and size can be evaluated by taking advantage of the sometimes significant differences in inspiratory vs. expiratory images, which may be useful in differentiating thymic hyperplasia from thymic masses.[29] Atrophic changes within the thymus, with replacement by fat, may be similarly subtle. When evaluating atrophic changes, stress-related atrophy and apoptosis may influence findings via imaging and microscopic evaluation. In experimental situations, the use of age-matched

controls held in similar situations and subjected to similar procedures, may reduce or eliminate this variable.

Hyperplastic lesions within the thymus may be the result of lymphoid follicular hyperplasia or true hyperplasia.[29] Lymphoid follicular hyperplasia involves irregular formation and proliferation of lymphoid follicles, with germinal center formation, and increased numbers of lymphocytes. In these cases, there may be other changes including areas of atrophy and reactive hyperplasia of the epithelium. True hyperplasia, or diffuse hyperplasia, is much less common, and grossly manifests as symmetrical, diffuse enlargement. In some cases, the enlargement can be severe, and may be associated with thyrotoxicosis, Graves' disease, and acromegaly.[195] Similar, marked increases in thymic size have been noted in calves repeatedly immunized with endotoxin.[215] Thymic rebound or regeneration following disease or injury, represents a thymic hyperplasia and must be considered in healing or recovering patients as a cause of increased thymic size or density.[29] Thymic rebound following treatment is useful as a prognostic indicator and indicative of good immune response.[149] In contrast, some cases of thymic lymphoid hyperplasia may be characterized by normal size and weight of the gland, and retention of normal shape.[191, 195]

Neoplastic processes also occur within the thymus. These are broadly classified as arising from either the lymphoid or epithelial cell components. Thymic lymphomas are particularly common in cats and young cattle. In cats, this may be in association with retroviral infection by feline leukemia virus (FeLV). Thymomas are defined as tumors arising from the epithelial cells of the thymus, and may be associated with non-neoplastic proliferation of lymphoid cells. Thymomas can be classified as predominantly

lymphocytic, predominantly epithelial, or mixed (lymphoepithelial), based on the proportion of the respective cell populations.[106] Thymomas are often benign, but can be invasive and malignant. Although CT scans are considered useful for detecting thymomas, invasion and metastasis are best detected via MRI or PET scans. In particular, superior contrast resolution and production of images in multiple planes, makes MRI especially useful for detecting local invasion.[38] Interestingly, one-third to one-half of human patients with thymoma develop myasthenia gravis,[29] an autoimmune disorder, in which antibodies block or alter acetycholine receptors at the neuromuscular junction.[116] Myasthenia gravis is also associated with thymic hyperplasia.[38] While the exact mechanisms and underlying pathogenesis which links these lesions is uncertain, it highlights the interrelatedness and importance of immune functions and autoimmunity in relation to thymic disease.

Imaging of the thymus often occurs in conjunction with histologic examination, particularly when an infectious, autoimmune, or neoplastic process is suggested. Histologic evaluation is also useful in therapeutic or experimental settings, in which findings due to the administration of pharmacological agents often correlate with thymic weight and peripheral lymphocyte counts.[61] Histology is necessary to differentiate hyperplastic vs. neoplastic lesions, to define distinct neoplastic entities (such as lymphoma vs thymoma), or determine the etiology of infectious diseases. Some diseases have characteristic signs or lesions, such as viral inclusion bodies, or intralesional protozoal/parasitic agents, but many times thymic lesions are nonspecific and associated with the similar end result of lymphoid apoptosis or lymphocytolysis. However, lesions and clinical signs in certain species, in conjunction with ancillary diagnostic tests, such as

bacterial culture, virus isolation, PCR, immunohistochemistry, or ELISA, may provide additional criteria by which diagnosis is made. Unfortunately, biopsies required for histopathologic examination create lesions of their own, complicating their use for multiple examinations and sample collections over time.

Radiation-Induced Injury

Ionizing radiation can result in immunosuppression, as lymphocytes and lymphoid progenitor cells throughout the body, including within the bone marrow, lymph nodes, spleen, and thymus, are damaged or killed. Radiomimetic drugs and chemotherapeutic agents can cause similar changes, as lymphocytes are sensitive to these as well. Ionizing radiation causes the generation of highly reactive free radicals, such as hydroxyl and hydrogen free radicals from water. Oxidative stress and generation of free radicals is an important cause of cell injury and death. These free radicals are unstable and are damaging to cells, causing lipid peroxidation of membranes, membrane destabilization, oxidative modification or fragmentation of proteins, and DNA damage.[42, 240] The apoptotic mechanism of cell death usually occurs within a few hours of radiation, and is followed by a second mechanism of radiation-induced injury, in which there is failure of mitosis and the inhibition of cellular proliferation.[183] However, the type, source, dose, intensity, and duration of exposure all play a role in determining the types of effects seen.

The x-rays and γ-rays of electromagnetic, ionizing radiation are most damaging to proliferating cells, which includes lymphoid and hematopoietic cells.[184] Other highly sensitive cells include any population that normally has a high rate of cellular turnover, such as mucosal epithelial cells throughout the body, including those within the

gastrointestinal system or mucous membranes. Some cells become necrotic rather quickly, while others undergo apoptotic pathways secondary to DNA damage, so that failure of mitosis in dividing cells may lead to cell death and activation of apoptotic pathways in interphase cells and differentiated cells.[183] Lesions which result may be acute or chronic in nature. Even single, moderate to severe radiation-exposure events may have long-term effects, due to cell or tissue loss, scarring, DNA repair dysfunction, or loss of proliferation control and the development of neoplasia.[193] There is a well-known link between ultraviolet (UV) radiation and the development of neoplastic skin diseases.[168]

In cases of high dose or penetrating, whole- to partial-body radiation, particularly that received in a short amount of time, acute radiation sickness can develop.[105, 244] Signs vary according to dose, degree, and location of exposure, but consist of gastrointestinal disturbances, cerebrovascular dysfunction, and hematopoietic changes, including lymphopenia, granulocytopenia, or thrombocytopenia. The resulting lymphopenia is especially common, and occurs in a predictable manner, often before the onset of other types of cytopenias.[244] At very high doses, radiation causes death relatively quickly, primarily due to neurological and cardiovascular breakdown. Intermediate doses cause gastrointestinal failure within several days. Lower doses may cause death within one to few weeks, primarily due to hematopoietic failure and immunosuppression.[221] To combat these problems in therapeutic uses or sub-lethal experimental studies, small and/or targeted doses of radiation effectively avoid acute to subacute radiation sickness and result in reduced to subclinical and desired effects.

Radiation-Induced Injury in the Thymus

Following x-irradiation, the thymus undergoes rapid decrease in size, caused by degradation of lymphoid tissues and cell death.[81] Cell destruction is due to apoptosis, characterized by extensive cell and nuclear fragmentation and nuclear pyknosis, as detected by cytofluorometric determination and TUNEL procedures.[179] Histologic evaluation of the thymus with radiation-induced injury shows selective depletion of lymphocytes, as these are the most sensitive cell population, while mostly sparing the epithelial and stromal components. There may be preservation and increased prominence of Hassell's corpuscles, due to the relative decrease in lymphoid cells. Additionally, there is reduction or loss of the corticomedullary distinction and overall disruption of tissue architecture. With more severe or prolonged radiation exposure, there is a wider range and increased numbers of cells affected, including stromal components and compromise of the vascular system.

Interestingly, the detrimental effects of ionizing radiation have been alleviated by pretreatment with protective cytokines, while the administration of other cytokines can have opposing and sensitizing effects.[161, 162] Mice given IL-1, IL-12, stem cell factor (SCF), and TNF were protected from the lethal effects of whole-body radiation. Conversely, mice treated with antibodies directed against these same cytokines had increased mortality.[163, 164] These cytokines also have been shown to promote recovery when administered after low doses of radiation.[163] The protective effects seem to be due to stimulation of cell cycling within progenitor or stem cell populations, prevention of apoptosis, and reduction of oxidative damage injury through induction of scavenging proteins or enzymes such as mitochondrial superoxide dismutase (SOD).[162, 221] There are

pitfalls to the usage of these cytokines, however, as they exhibit wide-reaching effects, and may have deleterious or unwanted side effects throughout multiple tissues or organ systems. Some cytokines, such as TNF-α or IL-12, seem to have simultaneous beneficial and harmful effects, with sensitization of some tissues.

Feline Immunodeficiency Virus Infection

Feline immunodeficiency virus (FIV), a member of the family Retroviridae, is a naturally occurring feline lentivirus. FIV belongs to the same genus (*Lentivirinae*) of viruses as the human immunodeficiency virus (HIV) and immunodeficiency viruses in multiple other host species, including visna-maedi in sheep and equine infectious anemia virus in horses. Lentiviruses are known for being species-specific, producing life-long infections, and causing slowly progressive diseases. First isolated in California in 1987,[187] FIV occurs worldwide, representing a significant cause of morbidity and mortality within the feline population. Its prevalence varies geographically, but approximately 1.5 to 3 percent of domestic cats in the United States are infected with FIV,[8, 135] and numbers have been noted to rise to up to 25% in feral cat populations.[167]

FIV causes severe depletion of T helper lymphocytes and eventually gives rise to a state of immunocompromise, immune dysfunction, and an acquired immunodeficiency syndrome (AIDS), similar to that noted in people infected with HIV.[14, 37, 63] With the decline of the immune system, there is a marked increase in susceptibility to opportunistic bacterial, viral, protozoal, or parasitic secondary infections. There is also an increased incidence of neoplasia, particularly lymphoid malignancies.[16, 17, 82, 220] AIDS-like syndromes in cats are often characterized by signs such as wasting, gingivitis and stomatitis, upper respiratory disease, skin infections, or neuropathies. The lifespan of

FIV-infected cats is highly variable, and more than 50% of FIV-infected cats remain asymptomatic for years.[8] However, about 20% of FIV-infected cats die within 2 years of diagnosis, or approximately 4 to 6 years after infection.

Clinical diagnosis of infection is most often via an antibody test, in which an enzyme-linked immuno-sorbent assay (ELISA) is used for screening.[94, 134] Confirmation of infection can be assessed through Western blot analysis or polymerase chain reaction (PCR). It must be taken into account, however, that detectable antibody levels are often not reached until 8-12 weeks post-infection, and that kittens may acquire passively transferred antibodies from the mother, so that retesting is required to rule out false-negatives and false positives, respectively.[107, 134]

Role as Animal Model

FIV has been proposed as an animal model for HIV, as the pathogenesis of FIV infection is similar to that seen in human cases of HIV, despite differences between the two viruses. Further support for FIV infection in cats as an animal model for the human disease is the close phylogenetic relationship of the two viruses, similarity of host-virus interactions, including mode of infection and viral transmission, disease pathogenesis, and resultant immunopathology. In addition, the cat, as the smallest known natural host of a lentivirus, with its reduced size, ease of handling, and production of multiple-offspring litters, makes FIV infection an even more useful and effective model for the study of HIV.[19, 34, 55, 60]

The genome of FIV, like that of HIV and other retroviruses, consists of single-stranded RNA, which uses the viral-encoded enzyme, reverse transcriptase, and the host cell's machinery, to produce a double-stranded DNA copy of the viral RNA.[60] The

double-stranded DNA copy of the viral genome is then inserted, or integrated, into the DNA of the host cell, where it is referred to as a provirus. Once integrated, the provirus may remain in an inactive, or latent, state for some time before production of new virus particles is initiated. [12, 233]

The morphology of the virion is typical of that of a lentivirus. A mature FIV particle is 100-125 nm in diameter and consists of an envelope, a nucleocapsid, and a nucleoid. It has a central, cone-shaped, electron-dense core and there are multiple short knobs or projections on the outer envelope.[15, 19, 256]

Disease Pathogenesis

FIV is transmitted via the transfer of bodily fluids, most often through deep, penetrating bite wounds, but may also be transmitted by blood transfusions, mucosal exposure, or through prenatal or postnatal vertical exposure.[174, 177, 199] Once infection occurs, there is an early viremia, detectable via PCR by days 10-14 post-infection.[58] The disease pattern which follows is commonly described as having three clinical stages and is modeled after a progression similar to that noted in HIV infection. Initially there is an acute phase, which lasts a few days to a few weeks, that may manifest clinically as a low-grade fever, lethargy, and lymphadenopathy, accompanied by anemia or leukopenia. This phase is subtle and likely often goes unnoticed by the owner.[36, 258] In this early stage of infection, the virus is carried to nearby lymph nodes, where it replicates in T lymphocytes.[62] The virus then spreads to other lymph nodes throughout the body, resulting in a generalized, but usually temporary, enlargement, in conjunction with a high level of circulating virus within the bloodstream.[18] Within the first few weeks of acute infection, the numbers of both $CD4^+$ and $CD8^+$ T lymphocytes decline.[257] There is initial

lymphopenia, but this is followed by a robust immune response characterized by the production of antibodies directed against the virus, suppression of circulating viral load, and a rebound in $CD8^+$ T lymphocytes.[134] This results in a reduction in the $CD4^+$:$CD8^+$ T lymphocyte ratio.

The second stage is an asymptomatic phase that may last for several years (Figure 3). Cats in this stage are considered carriers and may be infective to other cats, but usually have a relatively constant, low, often undetectable, level of viremia.[54, 147] During this phase, the inversion of the CD4:CD8 T lymphocyte ratio persists, and $CD8^+$ cell numbers may continue to increase slightly as $CD4^+$ T cells numbers progressively decline.[2, 115] However, over time, both $CD4^+$ and $CD8^+$ T-lymphocytes gradually begin to decline.[134]

The third stage occurs with the development of clinical signs due to the loss of $CD4^+$ T helper cells, declined immune function, and resultant immunocompromise.[14, 230] At this time there is increased susceptibility to opportunistic invaders that usually do not cause disease in normal, immunocompetent animals, resulting in chronic, recurrent, debilitating infections, myelosuppression, and an increased incidence of neoplasia.[153, 232] Death due to secondary infections may occur (Figure 3). This is similar to AIDS in those individuals infected with HIV. The development of immunodeficiency is associated with a depletion of $CD4^+$ lymphocytes within the blood and lymphoid tissues.[153] Several theories have been proposed to identify the mechanism of lymphocyte destruction, including direct viral cytopathogenicity, immune hyperactivation and exhaustion through chronic stimulation, inhibition of thymic output, bone marrow suppression, destruction of normal lymph node architecture, immune suppression mediated by viral and regulatory

gene products, or inappropriate immune destruction of uninfected cells.[224, 230] The exact cause is unknown, but it may be likely that a combination of methods act in concert to cause immune depletion and dysfunction. However, it is known that not only destruction of infected cells occurs, but also uninfected cell populations, as some studies have reported that while large numbers of peripheral blood mononuclear cells (PBMCs) underwent apoptosis, only a small fraction (<1%) of those were FIV-infected cells.[131]

Cytokine Involvement

Lentiviral infection induces alterations in cytokine production and responsiveness, and in turn, these cytokines can regulate viral replication and apoptosis of T lymphocytes.[139, 156] Alterations may include systemic, organ, or tissue-specific changes,[52, 53] such as may occur in the placenta,[41, 245] thymus,[137, 181] lymph nodes,[137] or brain.[30, 192] IL-2, which was originally named "T cell growth factor," protects lymphocytes from apoptosis, as well as protein oxidation and degradation. Without IL-2, T cells usually become anergic, or unresponsive, and undergo apoptosis. Studies show that there are increased numbers of T cells arrested in the G0/G1 phase of cell cycle in HIV patients, which proceed to apoptosis without the addition of IL-2.[33]

Early theories proposed that only activated, proliferating lymphocytes underwent FIV-infection driven apoptosis, such that a high percentage of apoptosis of lymphocytes in FIV-positive cats was chronologically related to entering the S-phase of the cell cycle.[208] It was suggested that certain signals associated with cell cycle progression into the S phase occurred in tandem with signals that primed the cell for apoptosis, due to abnormal cytokine signaling, such as increased levels of TNF-α,[152] or reduced responsiveness to IL-2.[208] There is evidence that as IL-2 and IFN-γ levels decrease,

caused by a decrease in Th1 type cells, there are relative increases in IL-4 and IL-10 levels, which are cytokines produced by Th2 cells. An imbalance of Th1 and Th2 responsiveness may thus play a role in the increased susceptibility of infected individuals to intracellular microbial infections.[1, 231]

Within the thymus, increased levels of IL-10, IL-12, and IFN- γ and decreased levels of IL-4 are noted with FIV-induced acute inflammatory lesions and lymphoid follicle formation.[51, 137] In general, there is a greater increase of Type 2 cytokines than Type 1 cytokines in the acutely infected thymus.

Fetal and Neonatal FIV Infection

Understanding the transfer of FIV from mother to the fetus or neonate, as a small animal model for HIV infection, may lead to the development of therapeutic agents and research strategies aimed at the prevention and amelioration of lentiviral disease in neonates. To better combat vertical transmission, the mechanisms by which it occurs and its deleterious effects must be understood. Determination of the time of greatest risk will also direct the ease at which vertical transmission may be interrupted. Preventive therapies would be most difficult earliest in gestation and relatively more feasible intrapartum or postpartum.[165]

Transmission of FIV, as in HIV, is known to occur during pregnancy,[43, 59, 144, 175] parturition,[59, 128] or postnatally via colostrum or milk.[3, 4, 175, 217] *In utero* transmission may occur through cell to cell transfer of virus through the placental tissues or via contamination of fetal blood and tissues by infected maternal mononuclear cells. Possible mechanisms responsible for vertical transmission during the peripartum period include transplacental mixing of maternal blood into the fetal circulation during active labor or

absorption of the virus through the infant's immature digestive tract.[154] The likelihood of intrapartum transmission is supported by recovery of virus, both cell-associated and cell-free, from vaginal washes of infected mothers, throughout pregnancy and in the immediate postpartum period.[124, 175, 176]

Neonatal and pediatric infected patients often experience a shorter incubation time and more rapid disease progression and death than do infected adults.[83, 176] Most HIV-infected infants will have clinical symptoms of infection during the first year of life and up to 16% die before their fourth birthday because of rapid destruction of the immune system and development of opportunistic infections.[249] Those infected *in utero* also have a two-fold risk of progression to AIDS or death by 12 months of age as compared with those infected during childbirth.[49] FIV-infected kittens develop immune dysfunction and disease similar to that in adult cats, but those infected *in utero* or at birth tend to also exhibit rapid progression of disease and decreased viability postnatally.[34, 174] The thymus rapidly involutes, $CD4^+CD8^+$ and $CD4^+CD8^-$ cells are depleted,[253] and there is decreased ability to replenish diminished and dysfunctional peripheral T cells. Advanced maternal disease during pregnancy is also associated with faster disease progression in offspring infected during pregnancy or immediately following.[23]

It is possible that the HIV-induced changes in the fetal thymus contribute to immune rejection of the fetus, spontaneous abortion, or reproductive failure, through an imbalance of maternal and fetal Th1- and Th2-type cytokines.[219] There are trends for increased expression of Th1 cytokines, which are usually suppressed during normal pregnancy, and decreased expression of Th2 cytokines in the placentas of infected versus noninfected cats. Increased placental expression of IFN-γ and IL-1-β was significantly

associated with increased fetal resorption in infected animals.[245] Increased production of Th1 cytokines and a decrease in IL-10, a Th2 cytokine, has also been linked to spontaneous abortion and underweight infants born to HIV-infected women.[247] Hormonally mediated effects on the thymus and B cells during pregnancy may also contribute the generation of local and systemic lymphocyte and cytokine profiles.[247] In acutely FIV infected cats, elevations of IFN-γ, IL-12p40, IL-4, and IL-10, among others, have been measured in various tissues, including thymus, lymph nodes, and spleen.[53] Infection may additionally produce placentitis, thereby aiding in the transfer of virus from mother to fetus[219] via altered local cytokine levels and disruption in membrane and vascular integrity.

Effects on the Thymus

Kittens inoculated *in utero* may have acute, but transient, thymic atrophy, which can partially regenerate.[113] Thymus-to-body weight ratios may be initially decreased, with severe thymic cortical depletion, reduction of thymocyte numbers, and decreased corticomedullary distinction in those infected fetally, but this can rebound or begin to approach normal. In contrast, neonatally infected kittens have been noted to have progressive decline in thymus-to-body weight ratio, with moderate, gradual decline in cortical thymocyte density, loss of corticomedullary distinction, which may persist, as well as the formation of lymphoid follicles.[113] This has also been noted in SIV-infected rhesus macaque models of HIV, in which infected animals initially had dramatically increased levels of thymocyte depletion and apoptosis, with a rebound in thymocyte progenitor numbers and increased levels of cell proliferation in the following weeks.[255] Viral loads may vary, as *in situ* hybridization and immunohistochemistry reveal an

increased propensity to support viral mRNA and protein expression in the fetal inoculates, while those infected neonatally did not always have detectable viral levels, supporting the theory that kittens infected *in utero* are more likely to harbor productive infection.[113] Thymocytes as well as stromal cells and dendritic cells also appear capable of harboring productive infection.[114, 255] Viral effects on the developing fetal thymus, which is particularly vulnerable to infection, may result in enhanced pathogenicity and progression of disease, acute and profound thymic atrophy, and increased levels of viremia. The specific changes noted within the fetal versus neonatal thymus and timing of viral infection may reflect variability of cell types and subpopulations actively or productively infected strain-dependent viral cell tropism, and the regenerative capability of fetal stem cells and stromal support cells. This window of potential opportunity that exists for thymic regeneration may be useful in the therapeutic intervention of perinatal FIV and HIV infection, particularly in conjunction with imaging studies, as presented here.

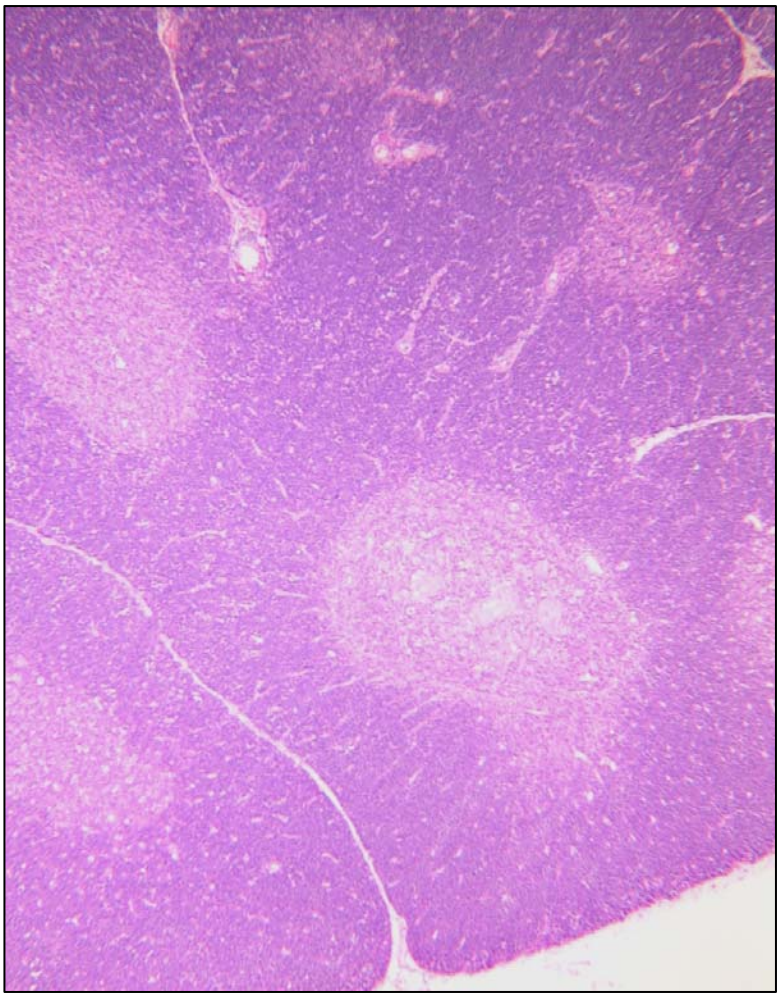

Figure 1. Histologic section (H&E stain) of the normal feline thymus, showing the relatively densely populated cortical region and the inner, relatively less densely populated medulla.

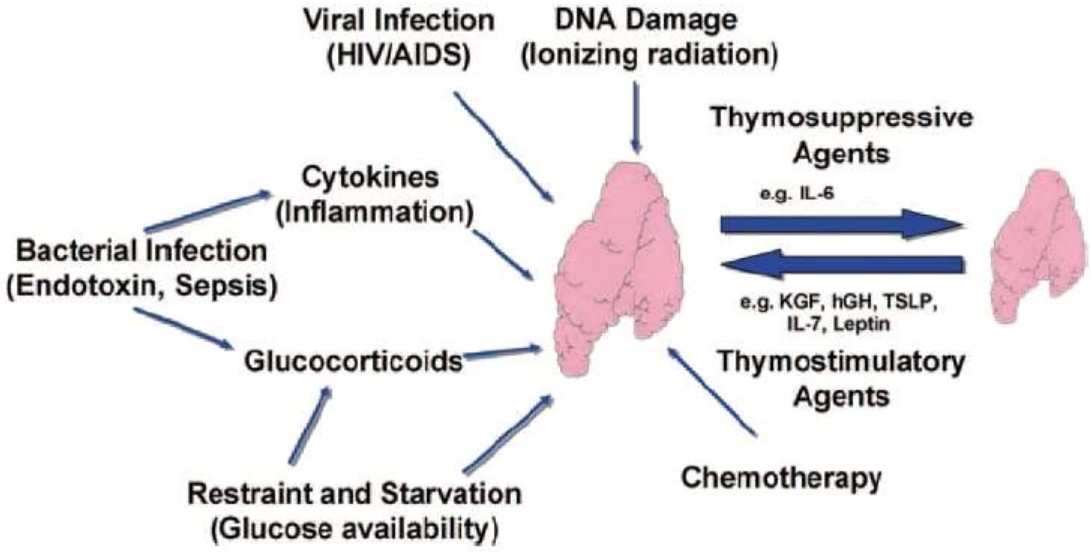

Figure 2. Influencesof stress and disease on the thymus. Modified from: Gruver AL and Sempowski GD, J Leukoc Biol, 2008.

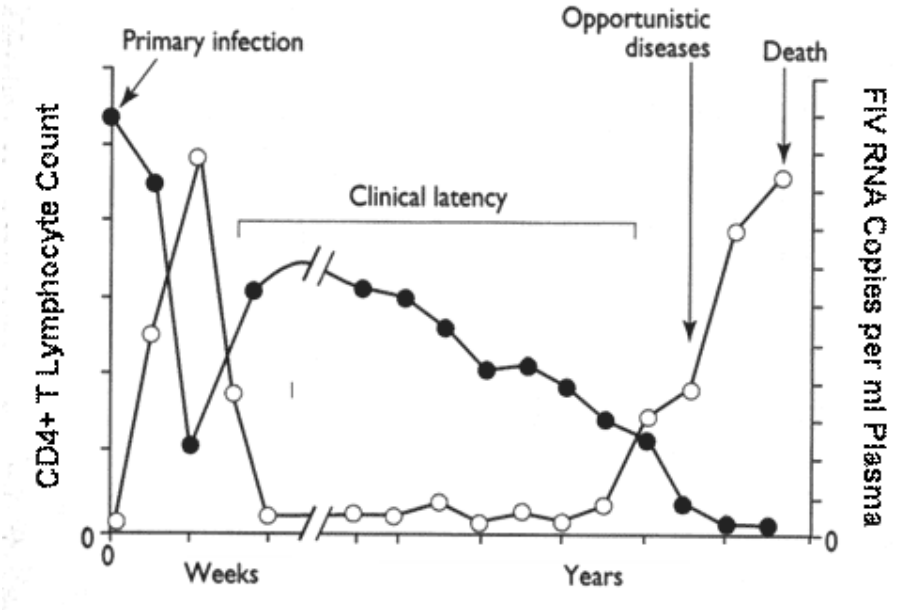

Figure 3. Progression of FIV disease. Modified from Fauci AS, Science, 1993.

II. MAGNETIC RESONANCE IMAGING OF RADIATION-INDUCED THYMIC ATROPHY AS A MODEL FOR PATHOLOGIC CHANGES IN ACUTE FELINE IMMUNODEFICIENCY VIRUS INFECTION

(Submitted to *Veterinary Radiology and Ultrasound*)

Abstract

Feline immunodeficiency virus (FIV) infection is characterized by progressive T lymphocyte depletion and dysfunction in association with severe thymic atrophy. The development of a protocol to reproducibly induce thymic atrophy, as occurs in FIV infection, and to consistently estimate thymic volume, would provide a valuable tool in the search of innovative and novel therapeutic strategies. Magnetic resonance imaging (MRI) using the short tau inversion recovery (STIR) technique, with fat suppression properties, was determined to provide an optimized means of locating, defining, and quantitatively estimating thymus volume. Thymic atrophy was induced in four, 8 to10-week-old kittens with a single, directed 500 cGy dose of 6 MV x-rays from a clinical linear accelerator, and sequential MR images of the cranial mediastinum were collected at 2, 7, 14, and 21 days post-irradiation (PI). Irradiation induced a severe reduction in thymic volume, which was decreased, on average, to 47% that of normal, by 7 days PI. Histopathology confirmed marked, diffuse thymic atrophy, characterized by reduced thymic volume, decreased overall cellularity, increased apoptosis, histiocytosis, and

reduced distinction of the cortico-medullary junction, comparable to that seen in acute FIV infection. Beginning on day 7 PI, thymic volumes rebounded slightly and continued to increase over the following 14 days, regaining 3-35% of original volume. These findings demonstrate the feasibility and advantages of using this non-invasive, *in vivo* imaging technique to measure and evaluate changes in thymic volume in physiologic and experimental situations.

Introduction

Feline immunodeficiency virus (FIV), of the family Retroviridae, is a naturally occurring lentivirus of cats. First isolated in California in 1987,[187] infection causes severe, progressive depletion and dysfunction of T helper lymphocytes, accompanied by thymic atrophy, and eventually gives rise to a state of immunocompromise and acquired immunodeficiency,[153] similar to that seen in human immunodeficiency virus (HIV) infection. FIV-induced thymic lesions include reduction in thymic volume and weight, decreased cellularity, increased apoptosis, and decreased distinction of the cortico-medullary junction.[48, 178] FIV infects approximately 1.5 to 3 % of domestic cats in the US[8] and up to 25% of certain feral cat populations worldwide.[167]

The thymus is uniquely and highly susceptible to immunodeficiency virus infection, particularly in neonatal or juvenile patients, at a time when the organ is physiologically most active.[95, 126, 253] It is thought that thymic atrophy and dysfunction are major contributors to pediatric or neonatal progression of disease, as those infected at an early age experience a shorter incubation time and rapid disease progression, as compared to those infected as adults.[83, 176] Located in the anterior mediastinum and caudal cervical region, the thymus is the primary lymphoid organ responsible for the

production of a diverse repertoire of immunocompetent and self tolerant T lymphocytes.[61, 111] In addition to a large circulating and continually migrating lymphoid cell population, this lobulated organ also consists of a significant epithelial and reticular stromal framework.[10, 85] Not only does this framework provide support through physical and direct cellular interactions, but it produces cytokines and other soluble factors, such as thymic stromal lymphopoietin (TSLP),[110, 120, 261] keratinocyte growth factor (KGF),[64, 70, 196] and IL-7,[79, 198, 209] that support thymopoiesis and orderly maturation of functional T lymphocytes. Upkeep of the normal thymic microenvironment and stromal support network is crucial to maintaining adequate T lymphocyte production and functional output. Disturbances of this network, as occurs in various infectious diseases, including lentiviral infections, can have dire consequences. However, there is often a period of partial regeneration and functional capacity following supportive and anti-retroviral therapy, which may be augmented by innovative new treatment modalities, including stem cell therapy and gene therapy.[6, 96, 190, 242]

In this study, we attempted to induce controlled thymic atrophy, mimicking changes as seen in acute FIV-infection, via external beam irradiation with x-rays, but in a highly consistent and reproducible manner. Thymic changes seen in natural or experimentally induced infections are subject to the variability of many intrinsic, host-specific factors, and the complex interactions of the host immune system with viral factors, such as viral strain, level of viremia, influence of mutations, disease status, and timing of infection.[35, 46, 47, 251] Minimizing these confounding factors allows for the greatest opportunity to study thymic atrophy and repair in a controlled manner and within a relatively finite time period. Ionizing radiation is known to produce organ- and tissue-

atrophy through direct cellular necrosis and induction of apoptosis.[117, 118, 228] Because of the high radiosensitivity of the thymus, rapid involution and regeneration of the thymus with sublethal γ -irradiation should occur.[67, 243] Because a single, targeted dose is delivered, there is expected to be a period of healing and regeneration of the thymus, particularly in young animals with significant regenerative capacity.[132, 142, 143] Measurement of thymic changes were monitored and measured using a MRI protocol developed specifically as a part of this study, in order to target enhanced visualization of the thymus. To the authors' knowledge, *in vivo* methods to quantify thymic volume with atrophic changes in the cat have not been published.

Materials and Methods

Pilot studies to determine optimum thymic imaging methods utilized a GE 9800 computed tomography unit (GE Medical Systems, Milwaukee, WI) and a Picker Vista 1.0 Tesla magnetic resonance unit (Picker, Cleveland, OH).

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Auburn University. Six, 8 to10-week old, clinically normal cats (8-035, 9-032, 10-784, and 10-787) were anesthetized by an intramuscular injection of ketamine (22mg/kg) and maintained with inhaled isoflurane (to effect) during all procedures. For magnetic resonance imaging, anesthetized cats were positioned in dorsal recumbency in a human extremity receiver coil, the front legs were extended, and images of the cranial mediastinum were made. Transverse plane images from the mid cervical area to the caudal aspect of the heart were obtained with a double spin echo sequence (TR 2425, TE 20/80, 192x256 matrix, 2 signal averages, 3 mm thick slices with 0 gap) and short tau inversion recovery (STIR) sequence (TR 2815, TE 30, TI 135, 192x256 matrix,

2 signal averages, 3 mm thick slice with 0 gap). Thymic atrophy was then induced in four of the six subjects with a single, directed 500 cGy dose of 6 MV x-rays from a Siemens clinical linear accelerator (Siemens Medical Solutions, Malvern, PA), with the beam collimated to the anterior mediastinum.

Magnetic resonance (MR) images were initially obtained immediately before irradiation, and at 2 (cats 8-035 and 9-032 only), 7, 14, and 21 days post-irradiation. Two clinically normal cats, H4-1 and H4-2, were used as age-matched controls, and were scanned at four, eight, and twelve weeks of age. All six cats in this study were male, with the exception of H4-1. Digital images of ten consecutive transverse magnetic resonance STIR slices were used to assess thymic size, beginning at the first image in which cranial lung tissue was visible and including the following 9 sections. ImageJ software, a public domain Java image processing program (National Institutes of Health, Besthesda, MD), was used to calculate area and pixel value statistics of an outline of the thymus in each successive slice. Cumulative pixilated areas were designated as the sum of the space occupied by the thymus in each of the ten serial images per subject.

For histologic evaluation, a 2-4 mm diameter section of anterior thymus was surgically collected 3 weeks post-irradiation (10-784, 10-787, and 8-035 only) and fixed in 4% paraformaldehyde. Thymic tissues were collected from non-irradiated subjects, for use as normal control tissue. Histologic sections were routinely prepared from fixed, paraffin-embedded tissue, sectioned at 4 μm thick, and stained with hematoxylin and eosin (H&E). These were compared to histologic sections of archival thymic tissue from age-matched, FIV-infected cats.

Non-parametric statistical analysis, utilizing the Kruskal-Wallis one-way ANOVA method was performed on pixel data, comparing the progression of thymic size over a finite period of time, as well as paired comparisons with the Wilcoxon signed-rank test.

Results

Pilot studies indicated that MR imaging, with STIR fat suppression parameters, was superior to CT imaging (data not shown). MR images provided more detail and sharper distinction of the thymus from adjacent, non-thymic connective tissue, including adipose tissue.

Analysis of MR images with the computer program ImageJ quantified the number of pixels in manually outlined thymus images (Figure 4A, inset). Thymic tissue was discernible within the mediastinum, of non-irradiated control subjects (Figure 4A-C) and irradiated subjects (Figure 5), particularly when contrasted with adjacent lung tissue. Cumulative pixel values were generated via assessment of multiple serial measurements, extending from the thoracic inlet to the base of the heart (Figure 5), in all subjects.

MR imaging from the two normal, non-irradiated subjects showed progressive and uniform enlargement of the thymus from four to twelve weeks of age, with increasing cumulative serial user-defined pixel values (Figure 4A-C, Table 1). Thymic measurements of subject H4-1 and H4-2 revealed a 35.05% and 75.08% increase in total thymic area at ages eight and twelve weeks, respectively, from that of the original scan at four weeks old. Thymic measurements of subject H4-2 yielded similar results, with a 27.15% and 33.95% increase in total thymic area at ages eight and twelve weeks,

respectively. There was a corresponding progressive increase in body weights for both animals during this time period.

Cumulative serial pixel values from all irradiated subjects were calculated at multiple points in time (Table 2). Total calculated pixel values for all irradiated subjects show a marked initial decline in thymic size at 2 to 7 days PI (Figures 6,7). Thymic size was decreased, on average, for all subjects, to 47.13 % that of the original area, at day 7 PI. This reduction in thymic area persisted through days 14 and 21 PI, however, the amount of the decrease, from that of the original area, was less severe by 21 days PI for all subjects, and less severe at day 14 PI for subjects 10-787 and 9-032. There was an overall, mild to moderate increase in thymic volume for all subjects from day 7 to day 21 PI. Thus, maximal size reduction was achieved by day 7 PI, but rebounded and continued to increase over the following 7 to 14 days, so that the subjects regained 3.23-35.16% of original size by day 21 PI.

Statistical analysis of the cumulative serial pixel values from irradiated subjects confirmed that the percentage of initial thymic volume was significantly different from subsequent post-irradiation measurements ($p = 0.032$). However, none of the paired comparisons were significantly different ($p=0.068$ to 0.72).

Histologic changes within the thymus confirmed that calculated measurement of the thymic volume corresponded with morphological changes following radiation treatment, as compared to the non-irradiated control subjects (Figure 8A). Within the thymus of irradiated subjects, there was marked reduction of thymic size, characterized by diffuse atrophy, with loss of cortical and medullary lymphocytes. There was decreased overall cellularity, loss of corticomedullary distinction, markedly increased numbers of

apoptotic cells, histiocytosis, and mild, multifocal, mixed inflammation (Figure 8B). This was in contrast to the normal, non-irradiated thymus of a similarly aged subject, in which there was a clear distinction of cortical and medullary zones, with a relatively high density of cells within the cortex, no inflammation, and few, scattered apoptotic cells.

Discussion

During FIV infection, neonatal and pediatric patients often experience a shorter incubation time and more rapid disease progression and death than do infected adults,[83, 176] with clinical signs of infection apparent during the first year of life due to the rapid destruction of the immune system and development of opportunistic infections.[249] FIV-infected kittens develop immune dysfunction and disease similar to that in adult cats, but those infected *in utero* or soon after birth have decreased viability in the postnatal and juvenile period.[34, 175] The thymus rapidly involutes, $CD4^+CD8^+$ and $CD4^+CD8$- cells are depleted,[253] and there is decreased ability to replenish diminished and dysfunctional peripheral T cells. These findings reiterate the critical importance of thymic function in the young animal, particularly in the face of disease and immune compromise.

Kittens inoculated *in utero* have been reported to have acute, but transient, thymic atrophy, which partially regenerated.[113] This was characterized by initially lowered thymus: body weight ratio, severe thymic cortical depletion, reduction of thymocyte numbers, and decreased corticomedullary distinction, but this later began to rebound or approach normal. This also has been noted in SIV-infected rhesus macaque models of HIV, in which infected animals initially had dramatically increased levels of thymocyte depletion and apoptosis, with a rebound in thymocyte progenitor numbers and increased levels of cell proliferation that occurred in the following weeks.[255] This reiterates the

potential for thymic rebound or regeneration, which could be augmented by treatment or therapeutic strategies.

In an effort to further characterize thymic morphologic and functional changes in health and disease, imaging data has proven to be a useful adjunct to research in multiple species.[29, 149] Insights gained from various imaging studies and the potential for regeneration of the thymus, may lead to new therapeutic protocols, and perhaps restoration of thymic function. This study was undertaken as a first step in a developing a systematic and non-invasive method by which to quantify changes in the volume of the feline thymus.

Reduction in thymic size was induced in all subjects receiving targeted mediastinal irradiation. Evaluation of successive MR images showed obvious visible changes in thymic size (Figure 6), which was corroborated by mathematical and statistical analysis (Table 2). Maximal atrophy was noted at 7 to14 days PI, although there was significant decrease in measured thymic area as rapidly as 2 days PI (Figure 7). By day 21 PI, there was a mild to moderate rebound in thymic size in all irradiated subjects. Given sufficient time, it is expected that the thymus would likely have returned to or approached normal or pre-irradiated size. No signs of immune dysfunction were noted in any of the subjects, and all were clinically normal for the duration of the study.

The size of the thymus in normal, non-irradiated control subjects increased steadily over time for the duration of the study (Figure 4, Table 1). Due to the young age of these animals, this likely represents physiologic expansion of the thymus prior to involution at puberty.

Histologic changes noted in the irradiated thymus were consistent with radiation-induced lymphocytolysis and overall loss of cellularity.[117, 118] There was marked decrease in thymic size and alteration of the normal thymic pattern of organization, with reduced numbers of cortical lymphocytes, numerous apoptotic cells, increased numbers of histiocytes, and mild inflammation (Figure 8). Although cell death and tissue destruction is achieved through different mechanisms in acutely FIV-infected cats, similar histologic changes can be noted. Fetal or neonatal kittens inoculated with FIV exhibited reduced thymocyte density, reduced corticomedullary distinction, architecture deformation and lymphoid follicle formation.[113, 169, 182]

Pilot studies conducted as a preliminary portion of this effort revealed an advantage of MRI as compared to CT images (data not shown) in the examined subjects, with enhanced visualization of the thymus. In particular, the fat suppression properties of STIR sequences were useful, even in the young subjects utilized in the study. As aging occurs, the thymus involutes as a normal physiologic process, in which the thymus is gradually replaced by and surrounded by increased amounts of adipose tissue.[84, 166] As the thymic volume decreases, the relative amount of adipose tissue increases. Interestingly, this change has been demonstrated as the result of chronic, age-related changes, as well as the result of more acute, infection-related or age-independent factors.[146] Therefore, even in these very young subjects with an active thymus, visualization and distinction of thymic tissue from adjacent adipose or connective tissues is essential and requires optimal image detail. MR imaging is a sensitive method for identifying closely associated fat and water in microscopic mixtures.[227] Additionally,

MRI provides superior detail of thymic margins,[223] which is particularly useful for measurement studies as conducted here.

Another advantage of utilizing MR imaging, is that it provides a non-invasive, non-terminal, *in vivo* means of evaluating the thymus in its normal anatomic location. It also allows for a longitudinal study, such as performed here, with evaluation of the same individual subject over a specified period of time. Such studies would control for the normal physiologic variability between individuals or groups due to breed, sex, nutritional status, or husbandry. This also minimizes confounding factors which may be introduced as the result of terminal, peri-mortem or postmortem changes and the effects of fixation and processing.

The results of this study could be augmented by larger sample sizes. Increased numbers of non-irradiated and irradiated subjects would provide more detailed and defined ranges of normal thymic volumes and insight as to the variation in image quality and definition due to the effects of age and/or irradiation. Due to physiologic changes and the dynamic nature of the thymus, studies over an extended period of time would also be beneficial. This could be coupled with ancillary information, such as peripheral blood and thymic lymphocyte subset counts and other indicators of thymic function.

In summary, MR imaging of the thymus utilizing short tau inversion recovery provided a useful estimate of thymic volume *in vivo*. Thymic atrophy was readily induced by external beam irradiation, which caused significant reduction in thymic size over 2-14 days following irradiation, but with subsequent histologic rebound and partial restoration of thymic size, evident by 21 days after irradiation. This experimental protocol will

facilitate longitudinal studies of thymus function and pathology in the cat, including comparative studies of immunosuppressive lentivirus infections.

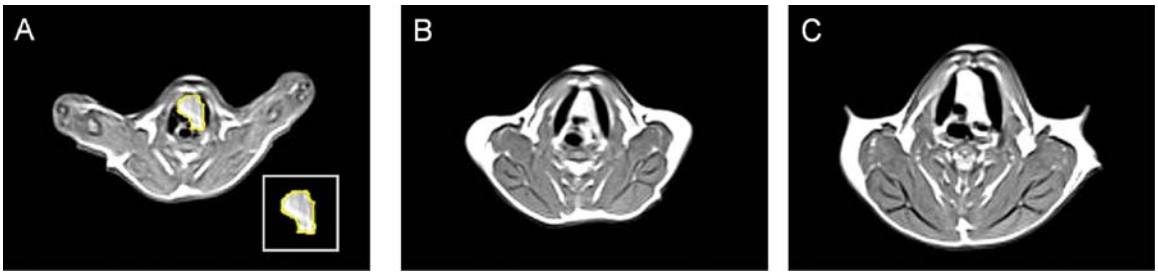

Figure 4. MR images of a single non-irradiated subject, with inset of outlined thymus, at 4 weeks (A), 8 weeks (B), and 12 weeks (C), of age. Outlined regions were used to generate cumulative pixel values for subjects, as an *in vivo* measurement of thymic size. All images are from approximately the same level of the cranial thorax.

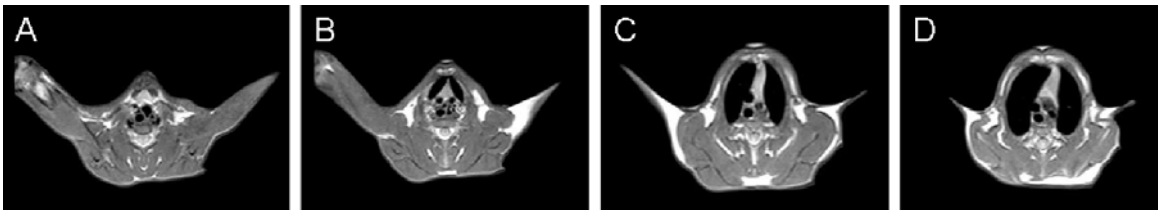

Figure 5. Successive images of the normal thymus, at 12 to 18 mm intervals, extending from the thoracic inlet (A), through the cranial mediastinum (B, C), to the heart base (D), in an eight-week-old subject prior to irradiation.

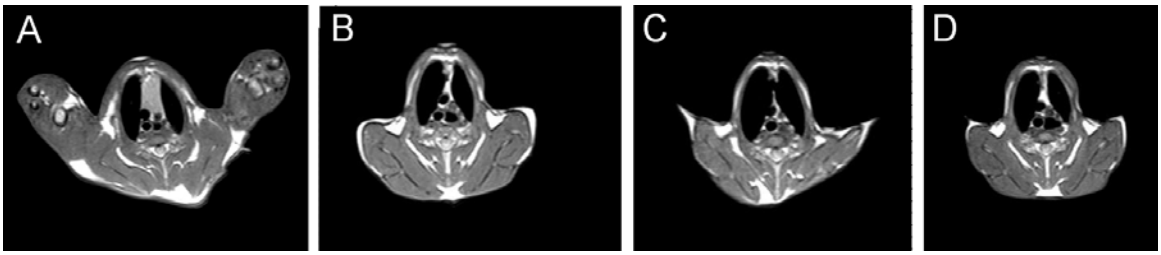

Figure 6. MR images of an irradiated subject, immediately prior to irradiation (A), and at 7 days (B), 14 days (C), and 21 days (D) post-irradiation (PI). All images are from approximately the same level of the cranial thorax. Note the progressive decrease in thymic size over the two weeks following radiation, followed by a mild increase in size, interpreted as thymic rebound or regeneration, at three weeks PI.

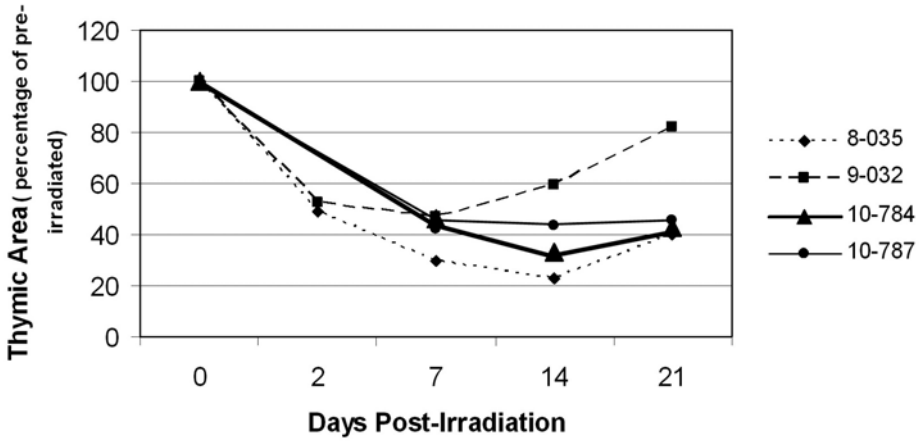

Figure 7. Change in measured thymic area over time, post-irradiation. Values are represented as the percentage of the original, pre-irradiation thymic size. Note the progressive decline in thymic size, beginning as early as 2 days PI, but with variable rebound or return of measured thymic area at 14 to 21 days PI.

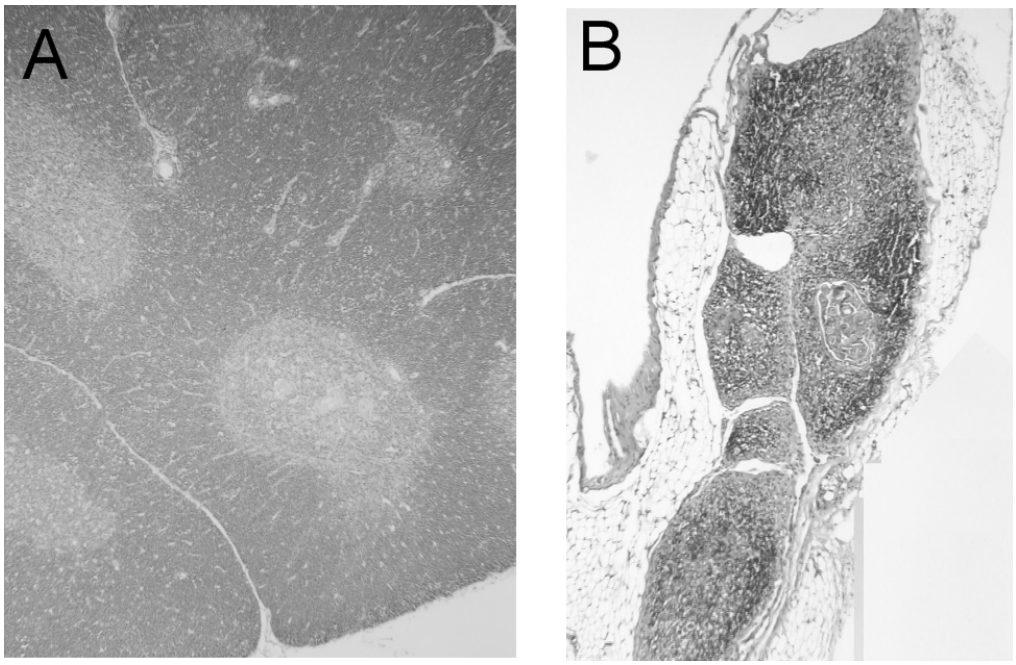

Figure 8. H&E stained thymic sections from age-matched normal (A) and irradiated (B) subjects. Note the marked reduction in thymic size, overall hypocellularity, and loss of corticomedullary distinction in the irradiated subject.

Table 1. Total Thymic Pixel Measurements for Non-Irradiated Controls

Subject	4 wks	8 wks	12 wks
H4-1	5086	6869	8905
H4-2	5868	7461	7860

Table 2. Thymic Pixel Measurements for Irradiated Subjects

		Total Calculated Pixelated Area				
Subject	Age at First Scan	Pre-Irradiation	2 days PI*	7 days PI*	14 days PI*	21 days PI*
9-032	10 wks	7891	4116	3698	4702	6472
8-035	10 wks	13728	6697	4109	3132	5515
10-784	8 wks	9146		4244	3002	3928
10-787	8 wks	7744		3259	3351	3539

* Post-Irradiation

LITERATURE CITED

1. Abbas A, Lichtman A and Pober J. Congenital and acquired immunodeficiencies. In:Cellular and Molecular Immunology. 4th ed.: W.B. Saunders Company, 2000; Ch. 20.

2. Ackley CD, Yamamoto JK, Levy N, Pedersen NC and Cooper MD. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. J Virol 1990;64:5652-5655.

3. Allison RW and Hoover EA. Covert vertical transmission of feline immunodeficiency virus. AIDS Res Hum Retroviruses 2003;19:421-434.

4. Allison RW and Hoover EA. Feline immunodeficiency virus is concentrated in milk early in lactation. AIDS Res Hum Retroviruses 2003;19:245-253.

5. Alpdogan O, Hubbard VM, Smith OM, et al. Keratinocyte growth factor (KGF) is required for postnatal thymic regeneration. Blood 2006;107:2453-2460.

6. Alpdogan O and van den Brink MR. IL-7 and IL-15: therapeutic cytokines for immunodeficiency. Trends Immunol 2005;26:56-64.

7. Alves NL, Arosa FA and van Lier RA. Common gamma chain cytokines: dissidence in the details. Immunol Lett 2007;108:113-120.

8. American Association of Feline Practitioners and the Cornell Feline Health Center CU, College of Veterinary Medicine. Feline Immunodeficiency Virus. http://www.vet.cornell.edu/fhc/brochures/fiv.html. 2008.

9. Anderson G, Jenkinson WE, Jones T, et al. Establishment and functioning of intrathymic microenvironments. Immunol Rev 2006;209:10-27.

10. Anderson G, Moore NC, Owen JJ and Jenkinson EJ. Cellular interactions in thymocyte development. Annu Rev Immunol 1996;14:73-99.

11. Aspinall R and Andrew D. Thymic involution in aging. J Clin Immunol 2000;20:250-256.

12. Assogba BD, Leavell S, Porter K and Burkhard MJ. Mucosal administration of low-dose cell-associated feline immunodeficiency virus promotes viral latency. J Infect Dis 2007;195:1184-1188.

13. Banwell CM, Partington KM, Jenkinson EJ and Anderson G. Studies on the role of IL-7 presentation by mesenchymal fibroblasts during early thymocyte development. Eur J Immunol 2000;30:2125-2129.

14. Barlough JE, Ackley CD, George JW, et al. Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections. J Acquir Immune Defic Syndr 1991;4:219-227.

15. Barr MC, Zou L, Holzschu DL, et al. Isolation of a highly cytopathic lentivirus from a nondomestic cat. J Virol 1995;69:7371-7374.

16. Beatty JA, Callanan JJ, Terry A, Jarrett O and Neil JC. Molecular and immunophenotypical characterization of a feline immunodeficiency virus (FIV)-associated lymphoma: a direct role for FIV in B-lymphocyte transformation? J Virol 1998;72:767-771.

17. Beatty JA, Lawrence CE, Callanan JJ, et al. Feline immunodeficiency virus (FIV)-associated lymphoma: a potential role for immune dysfunction in tumourigenesis. Vet Immunol Immunopathol 1998;65:309-322.

18. Beebe AM, Dua N, Faith TG, Moore PF, Pedersen NC and Dandekar S. Primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets. J Virol 1994;68:3080-3091.

19. Bendinelli M, Pistello M, Lombardi S, et al. Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. Clin Microbiol Rev 1995;8:87-112.

20. Beq S, Delfraissy JF and Theze J. Interleukin-7 (IL-7): immune function, involvement in the pathogenesis of HIV infection and therapeutic potential. Eur Cytokine Netw 2004;15:279-289.

21. Berger A. Th1 and Th2 responses: what are they? Bmj 2000;321:424.

22. Berry C. Anatomic and Physiologic Imaging of the Canine and Feline Brain. In:Textbook of Veterinary Diagnostic Radiology. 3rd ed. Philadelphia, PA: W.B. Saunders Company, 1998; 66-73.

23. Blanche S, Mayaux MJ, Rouzioux C, et al. Relation of the course of HIV infection in children to the severity of the disease in their mothers at delivery. N Engl J Med 1994;330:308-312.

24. Bodey B. Thymic reticulo-epithelial cells: key cells of neuroendocrine regulation. Expert Opin Biol Ther 2007;7:939-949.

25. Bodey B, Bodey B, Jr., Siegel SE and Kaiser HE. Involution of the mammalian thymus, one of the leading regulators of aging. In Vivo 1997;11:421-440.

26. Bodey B, Bodey B, Jr., Siegel SE and Kaiser HE. The role of the reticulo-epithelial (RE) cell network in the immuno-neuroendocrine regulation of intrathymic lymphopoiesis. Anticancer Res 2000;20:1871-1888.

27. Bodey B, Siegel SE and Kaiser HE. Restoration of the thymic cellular microenvironment following autologous bone marrow transplantation. In Vivo 2002;16:127-140.

28. Boesch C. Molecular aspects of magnetic resonance imaging and spectroscopy. Mol Aspects Med 1999;20:185-318.

29. Bogot NR and Quint LE. Imaging of thymic disorders. Cancer Imaging 2005;5:139-149.

30. Bragg DC, Hudson LC, Liang YH, Tompkins MB, Fernandes A and Meeker RB. Choroid plexus macrophages proliferate and release toxic factors in response to feline immunodeficiency virus. J Neurovirol 2002;8:225-239.

31. Budagian V, Bulanova E, Paus R and Bulfone-Paus S. IL-15/IL-15 receptor biology: a guided tour through an expanding universe. Cytokine Growth Factor Rev 2006;17:259-280.

32. Budavari AI, Whitaker MD and Helmers RA. Thymic hyperplasia presenting as anterior mediastinal mass in 2 patients with Graves disease. Mayo Clin Proc 2002;77:495-499.

33. Bull ME, Vahlenkamp TW, Dow JL, et al. Spontaneous T cell apoptosis in feline immunodeficiency virus (FIV)-infected cats is inhibited by IL2 and anti-B7.1 antibodies. Vet Immunol Immunopathol 2004;99:25-37.

34. Burkhard MJ and Dean GA. Transmission and immunopathogenesis of FIV in cats as a model for HIV. Curr HIV Res 2003;1:15-29.

35. Burkhard MJ, Mathiason CK, Bowdre T and Hoover EA. Feline immunodeficiency virus Gag- and Env-specific immune responses after vaginal versus intravenous infection. AIDS Res Hum Retroviruses 2001;17:1767-1778.

36. Callanan JJ. Feline immunodeficiency virus infection: a clinical and pathological perspective. In:Feline Immunology and Immunodeficiency. ed.: Oxford New York: University Press, 1995; 111-130.

37. Callanan JJ, Thompson H, Toth SR, et al. Clinical and pathological findings in feline immunodeficiency virus experimental infection. Vet Immunol Immunopathol 1992;35:3-13.

38. Camera L, Brunetti A, Romano M, Larobina M, Marano I and Salvatore M. Morphological imaging of thymic disorders. Ann Med 1999;31 Suppl 2:57-62.

39. Chantry D, Turner M and Feldmann M. Interleukin 7 (murine pre-B cell growth factor/lymphopoietin 1) stimulates thymocyte growth: regulation by transforming growth factor beta. Eur J Immunol 1989;19:783-786.

40. Chung B, Barbara-Burnham L, Barsky L and Weinberg K. Radiosensitivity of thymic interleukin-7 production and thymopoiesis after bone marrow transplantation. Blood 2001;98:1601-1606.

41. Coats KS. The feline immunodeficiency virus-infected cat: a model for lentivirus-induced placental immunopathology and reproductive failure (mini-review). Am J Reprod Immunol 2005;54:169-185.

42. Cotran R, Kumar V and Collins T. Robbins Pathologic Basis of Disease. Philadelphia, PA: W.B. Saunders Company, 1999.

43. Courgnaud V, Laure F, Brossard A, et al. Frequent and early in utero HIV-1 infection. AIDS Res Hum Retroviruses 1991;7:337-341.

44. Cox NR, Morrison NE, Sartin JL, Buonomo FC, Steele B and Baker HJ. Alterations in the growth hormone/insulin-like growth factor I pathways in feline GM1 gangliosidosis. Endocrinology 1999;140:5698-5704.

45. Crooks ME and Littman DR. Disruption of T lymphocyte positive and negative selection in mice lacking the CD8 beta chain. Immunity 1994;1:277-285.

46. de Parseval A, Ngo S, Sun P and Elder JH. Factors that increase the effective concentration of CXCR4 dictate feline immunodeficiency virus tropism and kinetics of replication. J Virol 2004;78:9132-9143.

47. de Rozieres S, Mathiason CK, Rolston MR, Chatterji U, Hoover EA and Elder JH. Characterization of a highly pathogenic molecular clone of feline immunodeficiency virus clade C. J Virol 2004;78:8971-8982.

48. de Rozieres S, Swan CH, Sheeter DA, et al. Assessment of FIV-C infection of cats as a function of treatment with the protease inhibitor, TL-3. Retrovirology 2004;1:38.

49. de Souza RS, Gomez-Marin O, Scott GB, et al. Effect of prenatal zidovudine on disease progression in perinatally HIV-1-infected infants. J Acquir Immune Defic Syndr 2000;24:154-161.

50. Dean GA, Barger A and Lavoy A. Cloning and expression of feline interleukin 15. Cytokine 2005;29:77-83.

51. Dean GA, Higgins J, LaVoy A, Fan Z and Pedersen NC. Measurement of feline cytokine gene expression by quantitative-competitive RT-PCR. Vet Immunol Immunopathol 1998;63:73-82.

52. Dean GA, LaVoy A, Yearley J and Stanton C. Cytokine modulation of the innate immune response in feline immunodeficiency virus-infected cats. J Infect Dis 2006;193:1520-1527.

53. Dean GA and Pedersen NC. Cytokine response in multiple lymphoid tissues during the primary phase of feline immunodeficiency virus infection. J Virol 1998;72:9436-9440.

54. Dean GA, Reubel GH, Moore PF and Pedersen NC. Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node. J Virol 1996;70:5165-5169.

55. Dias AS, Bester MJ, Britz RF and Apostolides Z. Animal models used for the evaluation of antiretroviral therapies. Curr HIV Res 2006;4:431-446.

56. Donskoy E, Foss D and Goldschneider I. Gated importation of prothymocytes by adult mouse thymus is coordinated with their periodic mobilization from bone marrow. J Immunol 2003;171:3568-3575.

57. Donskoy E and Goldschneider I. Thymocytopoiesis is maintained by blood-borne precursors throughout postnatal life. A study in parabiotic mice. J Immunol 1992;148:1604-1612.

58. Dua N, Reubel G, Moore PF, Higgins J and Pedersen NC. An experimental study of primary feline immunodeficiency virus infection in cats and a historical comparison to

acute simian and human immunodeficiency virus diseases. Vet Immunol Immunopathol 1994;43:337-355.

59. Ehrnst A, Lindgren S, Dictor M, et al. HIV in pregnant women and their offspring: evidence for late transmission. Lancet 1991;338:203-207.

60. Elder JH, Sundstrom M, de Rozieres S, de Parseval A, Grant CK and Lin YC. Molecular mechanisms of FIV infection. Vet Immunol Immunopathol 2008.

61. Elmore SA. Enhanced histopathology of the thymus. Toxicol Pathol 2006;34:656-665.

62. English RV, Johnson CM, Gebhard DH and Tompkins MB. In vivo lymphocyte tropism of feline immunodeficiency virus. J Virol 1993;67:5175-5186.

63. English RV, Nelson P, Johnson CM, Nasisse M, Tompkins WA and Tompkins MB. Development of clinical disease in cats experimentally infected with feline immunodeficiency virus. J Infect Dis 1994;170:543-552.

64. Erickson M, Morkowski S, Lehar S, et al. Regulation of thymic epithelium by keratinocyte growth factor. Blood 2002;100:3269-3278.

65. Fabris N, Mocchegiani E, Mariotti S, Pacini F and Pinchera A. Thyroid function modulates thymic endocrine activity. J Clin Endocrinol Metab 1986;62:474-478.

66. Fabris N, Mocchegiani E, Mariotti S, Pacini F and Pinchera A. Thyroid-thymus interactions during development and aging. Horm Res 1989;31:85-89.

67. Faltynek CR, Wang S, Miller D, et al. Administration of human recombinant IL-7 to normal and irradiated mice increases the numbers of lymphocytes and some immature cells of the myeloid lineage. J Immunol 1992;149:1276-1282.

68. Fehniger TA, Cooper MA and Caligiuri MA. Interleukin-2 and interleukin-15: immunotherapy for cancer. Cytokine Growth Factor Rev 2002;13:169-183.

69. Fehniger TA, Shah MH, Turner MJ, et al. Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response. J Immunol 1999;162:4511-4520.

70. Finch PW and Rubin JS. Keratinocyte growth factor/fibroblast growth factor 7, a homeostatic factor with therapeutic potential for epithelial protection and repair. Adv Cancer Res 2004;91:69-136.

71. Finkelman FD and Urban JF, Jr. The other side of the coin: the protective role of the TH2 cytokines. J Allergy Clin Immunol 2001;107:772-780.

72. Fisher AG, Burdet C, Bunce C, Merkenschlager M and Ceredig R. Lymphoproliferative disorders in IL-7 transgenic mice: expansion of immature B cells which retain macrophage potential. Int Immunol 1995;7:415-423.

73. Fisher AG, Burdet C, LeMeur M, Haasner D, Gerber P and Ceredig R. Lymphoproliferative disorders in an IL-7 transgenic mouse line. Leukemia 1993;7 Suppl 2:S66-68.

74. Foss DL, Donskoy E and Goldschneider I. The importation of hematogenous precursors by the thymus is a gated phenomenon in normal adult mice. J Exp Med 2001;193:365-374.

75. Foss DL, Donskoy E and Goldschneider I. Functional demonstration of intrathymic binding sites and microvascular gates for prothymocytes in irradiated mice. Int Immunol 2002;14:331-338.

76. Foxwell BM, Beadling C, Guschin D, Kerr I and Cantrell D. Interleukin-7 can induce the activation of Jak 1, Jak 3 and STAT 5 proteins in murine T cells. Eur J Immunol 1995;25:3041-3046.

77. Freedman BD, Liu QH, Gaulton G, Kotlikoff MI, Hescheler J and Fleischmann BK. ATP-evoked Ca2+ transients and currents in murine thymocytes: possible role for P2X receptors in death by neglect. Eur J Immunol 1999;29:1635-1646.

78. French RA, Broussard SR, Meier WA, et al. Age-associated loss of bone marrow hematopoietic cells is reversed by GH and accompanies thymic reconstitution. Endocrinology 2002;143:690-699.

79. Fry TJ and Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. J Immunol 2005;174:6571-6576.

80. Fry TJ, Moniuszko M, Creekmore S, et al. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. Blood 2003;101:2294-2299.

81. Fujikawa K, Hasegawa Y, Matsuzawa S, Fukunaga A, Itoh T and Kondo S. Dose and dose-rate effects of X rays and fission neutrons on lymphocyte apoptosis in p53(+/+) and p53(-/-) mice. J Radiat Res (Tokyo) 2000;41:113-127.

82. Gabor LJ, Love DN, Malik R and Canfield PJ. Feline immunodeficiency virus status of Australian cats with lymphosarcoma. Aust Vet J 2001;79:540-545.

83. Galli L, de Martino M, Tovo PA, et al. Onset of clinical signs in children with HIV-1 perinatal infection. Italian Register for HIV Infection in Children. Aids 1995;9:455-461.

84. Garcia-Suarez O, Perez-Perez M, Germana A, Esteban I and Germana G. Involvement of growth factors in thymic involution. Microsc Res Tech 2003;62:514-523.

85. Gill J, Malin M, Sutherland J, Gray D, Hollander G and Boyd R. Thymic generation and regeneration. Immunol Rev 2003;195:28-50.

86. Giovanella L, Suriano S and Ceriani L. Graves' disease, thymus enlargement, and hypercalcemia. N Engl J Med 2008;358:1078-1079.

87. Godfrey DI, Kennedy J, Suda T and Zlotnik A. A developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8- triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. J Immunol 1993;150:4244-4252.

88. Gosain A and Gamelli RL. A primer in cytokines. J Burn Care Rehabil 2005;26:7-12.

89. Grencis RK. Th2-mediated host protective immunity to intestinal nematode infections. Philos Trans R Soc Lond B Biol Sci 1997;352:1377-1384.

90. Grossman CJ. Interactions between the gonadal steroids and the immune system. Science 1985;227:257-261.

91. Gruver AL, Hudson LL and Sempowski GD. Immunosenescence of ageing. J Pathol 2007;211:144-156.

92. Gruver AL and Sempowski GD. Cytokines, leptin, and stress-induced thymic atrophy. J Leukoc Biol 2008.

93. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123-1132.

94. Hartmann K. Feline immunodeficiency virus infection: an overview. Vet J 1998;155:123-137.

95. Haynes BF and Hale LP. The human thymus. A chimeric organ comprised of central and peripheral lymphoid components. Immunol Res 1998;18:61-78.

96. Hazra R and Mackall C. Thymic function in HIV infection. Curr HIV/AIDS Rep 2005;2:24-28.

97. Heinzel K, Benz C, Martins VC, Haidl ID and Bleul CC. Bone marrow-derived hemopoietic precursors commit to the T cell lineage only after arrival in the thymic microenvironment. J Immunol 2007;178:858-868.

98. Henson SM, Pido-Lopez J and Aspinall R. Reversal of thymic atrophy. Exp Gerontol 2004;39:673-678.

99. Herranz M, Martin-Caballero J, Fraga MF, et al. The novel DNA methylation inhibitor zebularine is effective against the development of murine T-cell lymphoma. Blood 2006;107:1174-1177.

100. Hoa D and Micheau A. e-MRI, Magnetic Resonance Imaging physics and technique course on the web. http://www.e-mri.org/. 2007.

101. Huang J and Muegge K. Control of chromatin accessibility for V(D)J recombination by interleukin-7. J Leukoc Biol 2001;69:907-911.

102. Huang M, Sharma S, Zhu LX, et al. IL-7 inhibits fibroblast TGF-beta production and signaling in pulmonary fibrosis. J Clin Invest 2002;109:931-937.

103. Hulstaert F, Hannet I, Deneys V, et al. Age-related changes in human blood lymphocyte subpopulations. II. Varying kinetics of percentage and absolute count measurements. Clin Immunol Immunopathol 1994;70:152-158.

104. Imamura T, Imamura T, Maeda H, et al. Cloning of full length cDNA, high-level expression and biological characterization of feline IL-12. J Vet Med Sci 2000;62:1079-1087.

105. Jackson WL, Jr., Gallagher C, Myhand RC and Waselenko JK. Medical management of patients with multiple organ dysfunction arising from acute radiation syndrome. BJR Suppl 2005;27:161-168.

106. Jacobs R, Messick J and Valli V. Tumors of the hemolymphatic system. In:Tumors in domestic animals. 4th ed. Ames, IA: Iowa State University Press, 2002; 165-166.

107. Jacobson RH. How well do serodiagnostic tests predict the infection or disease status of cats? J Am Vet Med Assoc 1991;199:1343-1347.

108. Jamieson BD, Douek DC, Killian S, et al. Generation of functional thymocytes in the human adult. Immunity 1999;10:569-575.

109. Jenkinson EJ, Jenkinson WE, Rossi SW and Anderson G. The thymus and T-cell commitment: the right niche for Notch? Nat Rev Immunol 2006;6:551-555.

110. Jiang Q, Coffield VM, Kondo M and Su L. TSLP is involved in expansion of early thymocyte progenitors. BMC Immunol 2007;8:11.

111. Jin R, Zhang J and Chen W. Thymic output: influence factors and molecular mechanism. Cell Mol Immunol 2006;3:341-350.

112. Jin Y and Wu MX. Requirement of Galphai in thymic homing and early T cell development. Mol Immunol 2008;45:3401-3410.

113. Johnson CM, Bortnick SJ, Crawford PC and Papadi GP. Unique susceptibility of the fetal thymus to feline immunodeficiency virus infection: an animal model for HIV infection in utero. Am J Reprod Immunol 2001;45:273-288.

114. Johnson CM, Papadi GP, Tompkins WA, et al. Biphasic thymus response by kittens inoculated with feline immunodeficiency virus during fetal development. Vet Pathol 1998;35:191-201.

115. Joshi A, Vahlenkamp TW, Garg H, Tompkins WA and Tompkins MB. Preferential replication of FIV in activated CD4(+)CD25(+)T cells independent of cellular proliferation. Virology 2004;321:307-322.

116. Juel VC and Massey JM. Myasthenia gravis. Orphanet J Rare Dis 2007;2:44.

117. Kakizaki T, Hamada N, Sakashita T, et al. Vulnerability of feline T-lymphocytes to charged particles. J Vet Med Sci 2007;69:605-609.

118. Kakizaki T, Hamada N, Wada S, et al. Distinct modes of cell death by ionizing radiation observed in two lines of feline T-lymphocytes. J Radiat Res (Tokyo) 2006;47:237-243.

119. Kanegane H and Tosato G. Activation of naive and memory T cells by interleukin-15. Blood 1996;88:230-235.

120. Kang J and Der SD. Cytokine functions in the formative stages of a lymphocyte's life. Curr Opin Immunol 2004;16:180-190.

121. Kaplan RN, Psaila B and Lyden D. Niche-to-niche migration of bone-marrow-derived cells. Trends Mol Med 2007;13:72-81.

122. Kendall MD, Fitzpatrick FT, Greenstein BD, Khoylou F, Safieh B and Hamblin A. Reversal of ageing changes in the thymus of rats by chemical or surgical castration. Cell Tissue Res 1990;261:555-564.

123. Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 2003;8:223-246.

124. Kiessling AA. Isolation of human immunodeficiency virus type 1 from semen and vaginal fluids. Methods Mol Biol 2005;304:71-86.

125. Kolaeva SG, Novoselova EG, Amerkhanov ZG, Kulikov AV and Ivkov VG. [The annual involution and regeneration of the thymus in hibernating animals and perspectives of its studies in gerontology and stem cell proliferation]. Tsitologiia 2003;45:628-634.

126. Kolenda-Roberts HM, Kuhnt LA, Jennings RN, Mergia A, Gengozian N and Johnson CM. Immunopathogenesis of feline immunodeficiency virus infection in the fetal and neonatal cat. Front Biosci 2007;12:3668-3682.

127. Krueger A and von Boehmer H. Identification of a T lineage-committed progenitor in adult blood. Immunity 2007;26:105-116.

128. Kuhn L, Steketee RW, Weedon J, et al. Distinct risk factors for intrauterine and intrapartum human immunodeficiency virus transmission and consequences for disease progression in infected children. Perinatal AIDS Collaborative Transmission Study. J Infect Dis 1999;179:52-58.

129. Kwissa M, Kroger A, Hauser H, Reimann J and Schirmbeck R. Cytokine-facilitated priming of CD8+ T cell responses by DNA vaccination. J Mol Med 2003;81:91-101.

130. Lamberts SW, van den Beld AW and van der Lely AJ. The endocrinology of aging. Science 1997;278:419-424.

131. Lawson M, Meers J, Blechynden L, Robinson W, Greene W and Carnegie P. The detection and quantification of feline immunodeficiency provirus in peripheral blood mononuclear cells using the polymerase chain reaction. Vet Microbiol 1993;38:11-21.

132. Legrand N, Dontje W, van Lent AU, Spits H and Blom B. Human thymus regeneration and T cell reconstitution. Semin Immunol 2007;19:280-288.

133. Leite de Moraes MC, Hontebeyrie-Joskowicz M, Leboulenger F, Savino W, Dardenne M and Lepault F. Studies on the thymus in Chagas' disease. II. Thymocyte subset fluctuations in Trypanosoma cruzi-infected mice: relationship to stress. Scand J Immunol 1991;33:267-275.

134. Levy J, Crawford C, Hartmann K, et al. 2008 American Association of Feline Practitioners' feline retrovirus management guidelines. J Feline Med Surg 2008;10:300-316.

135. Levy JK, Scott HM, Lachtara JL and Crawford PC. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. J Am Vet Med Assoc 2006;228:371-376.

136. Li L, Hsu HC, Stockard CR, et al. IL-12 inhibits thymic involution by enhancing IL-7- and IL-2-induced thymocyte proliferation. J Immunol 2004;172:2909-2916.

137. Liang Y, Hudson LC, Levy JK, Ritchey JW, Tompkins WA and Tompkins MB. T cells overexpressing interferon-gamma and interleukin-10 are found in both the thymus and secondary lymphoid tissues of feline immunodeficiency virus-infected cats. J Infect Dis 2000;181:564-575.

138. Lind EF, Prockop SE, Porritt HE and Petrie HT. Mapping precursor movement through the postnatal thymus reveals specific microenvironments supporting defined stages of early lymphoid development. J Exp Med 2001;194:127-134.

139. Linenberger ML and Deng T. The effects of feline retroviruses on cytokine expression. Vet Immunol Immunopathol 1999;72:343-368.

140. Lutz NW, Tome ME, Aiken NR and Briehl MM. Changes in phosphate metabolism in thymoma cells suggest mechanisms for resistance to dexamethasone-induced apoptosis. A 31P NMR spectroscopic study of cell extracts. NMR Biomed 2002;15:356-366.

141. Ma J, Kennedy-Stoskopf S, Sellon R, et al. Tumor necrosis factor-alpha responses are depressed and interleukin-6 responses unaltered in feline immunodeficiency virus infected cats. Vet Immunol Immunopathol 1995;46:35-50.

142. Mackall CL and Gress RE. Thymic aging and T-cell regeneration. Immunol Rev 1997;160:91-102.

143. Mackall CL, Punt JA, Morgan P, Farr AG and Gress RE. Thymic function in young/old chimeras: substantial thymic T cell regenerative capacity despite irreversible age-associated thymic involution. Eur J Immunol 1998;28:1886-1893.

144. Mandelbrot L, Brossard Y, Aubin JT, et al. Testing for in utero human immunodeficiency virus infection with fetal blood sampling. Am J Obstet Gynecol 1996;175:489-493.

145. Mantuano-Barradas M, Henriques-Pons A, Araujo-Jorge TC, Di Virgilio F, Coutinho-Silva R and Persechini PM. Extracellular ATP induces cell death in

CD4+/CD8+ double-positive thymocytes in mice infected with Trypanosoma cruzi. Microbes Infect 2003;5:1363-1371.

146. Marinova TT. Epithelial framework reorganization during human thymus involution. Gerontology 2005;51:14-18.

147. Matteucci D, Baldinotti F, Mazzetti P, et al. Detection of feline immunodeficiency virus in saliva and plasma by cultivation and polymerase chain reaction. J Clin Microbiol 1993;31:494-501.

148. McGhee JR. The world of TH1/TH2 subsets: first proof. J Immunol 2005;175:3-4.

149. Mendelson DS. Imaging of the thymus. Chest Surg Clin N Am 2001;11:269-293, x.

150. Miosge L and Zamoyska R. Signalling in T-cell development: is it all location, location, location? Curr Opin Immunol 2007;19:194-199.

151. Misslitz A, Pabst O, Hintzen G, et al. Thymic T cell development and progenitor localization depend on CCR7. J Exp Med 2004;200:481-491.

152. Mizuno T, Goto Y, Baba K, Masuda K, Ohno K and Tsujimoto H. TNF-alpha-induced cell death in feline immunodeficiency virus-infected cells is mediated by the caspase cascade. Virology 2001;287:446-455.

153. Mizuno T, Goto Y, Baba K, et al. Quantitative analysis of Fas and Fas ligand mRNAs in a feline T-lymphoid cell line after infection with feline immunodeficiency virus and primary peripheral blood mononuclear cells obtained from cats infected with the virus. Vet Immunol Immunopathol 2003;93:117-123.

154. Mohlala BK, Tucker TJ, Besser MJ, et al. Investigation of HIV in amniotic fluid from HIV-infected pregnant women at full term. J Infect Dis 2005;192:488-491.

155. Montecino-Rodriguez E, Clark R and Dorshkind K. Effects of insulin-like growth factor administration and bone marrow transplantation on thymopoiesis in aged mice. Endocrinology 1998;139:4120-4126.

156. Mortola E, Endo Y, Mizuno T, et al. Effect of interleukin-12 and interleukin-10 on the virus replication and apoptosis in T-cells infected with feline immunodeficiency virus. J Vet Med Sci 1998;60:1181-1185.

157. Nagy PV, Feher T, Morga S and Matko J. Apoptosis of murine thymocytes induced by extracellular ATP is dose- and cytosolic pH-dependent. Immunol Lett 2000;72:23-30.

158. Nakahara T, Fujii H, Ide M, et al. FDG uptake in the morphologically normal thymus: comparison of FDG positron emission tomography and CT. Br J Radiol 2001;74:821-824.

159. Napolitano LA, Schmidt D, Gotway MB, et al. Growth hormone enhances thymic function in HIV-1-infected adults. J Clin Invest 2008;118:1085-1098.

160. Nelson RP, Jr. and Ballow M. 26. Immunomodulation and immunotherapy: drugs, cytokines, cytokine receptors, and antibodies. J Allergy Clin Immunol 2003;111:S720-743.

161. Neta R. Modulation of radiation damage by cytokines. Stem Cells 1997;15 Suppl 2:87-94.

162. Neta R. Modulation with cytokines of radiation injury: suggested mechanisms of action. Environ Health Perspect 1997;105 Suppl 6:1463-1465.

163. Neta R and Oppenheim JJ. Radioprotection with cytokines--learning from nature to cope with radiation damage. Cancer Cells 1991;3:391-396.

164. Neta R, Oppenheim JJ, Schreiber RD, Chizzonite R, Ledney GD and MacVittie TJ. Role of cytokines (interleukin 1, tumor necrosis factor, and transforming growth factor beta) in natural and lipopolysaccharide-enhanced radioresistance. J Exp Med 1991;173:1177-1182.

165. Newell ML. Mechanisms and timing of mother-to-child transmission of HIV-1. Aids 1998;12:831-837.

166. Nishino M, Ashiku SK, Kocher ON, Thurer RL, Boiselle PM and Hatabu H. The thymus: a comprehensive review. Radiographics 2006;26:335-348.

167. Norris JM, Bell ET, Hales L, et al. Prevalence of feline immunodeficiency virus infection in domesticated and feral cats in eastern Australia. J Feline Med Surg 2007;9:300-308.

168. Norval M. The mechanisms and consequences of ultraviolet-induced immunosuppression. Prog Biophys Mol Biol 2006;92:108-118.

169. Norway RM, Crawford PC, Johnson CM and Mergia A. Thymic lesions in cats infected with a pathogenic molecular clone or an ORF-A/2-deficient molecular clone of feline immunodeficiency virus. J Virol 2001;75:5833-5841.

170. Novoselova EG, Kolaeva SG, Makar VR and Agaphonova TA. Production of tumor necrosis factor in cells of hibernating ground squirrels Citellus undulatus during annual cycle. Life Sci 2000;67:1073-1080.

171. Nowell CS, Farley AM and Blackburn CC. Thymus organogenesis and development of the thymic stroma. Methods Mol Biol 2007;380:125-162.

172. O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. Immunity 1998;8:275-283.

173. O'Garra A, McEvoy LM and Zlotnik A. T-cell subsets: chemokine receptors guide the way. Curr Biol 1998;8:R646-649.

174. O'Neil LL, Burkhard MJ, Diehl LJ and Hoover EA. Vertical transmission of feline immunodeficiency virus. Semin Vet Med Surg (Small Anim) 1995;10:266-278.

175. O'Neil LL, Burkhard MJ, Diehl LJ and Hoover EA. Vertical transmission of feline immunodeficiency virus. AIDS Res Hum Retroviruses 1995;11:171-182.

176. O'Neil LL, Burkhard MJ and Hoover EA. Frequent perinatal transmission of feline immunodeficiency virus by chronically infected cats. J Virol 1996;70:2894-2901.

177. Obert LA and Hoover EA. Feline immunodeficiency virus clade C mucosal transmission and disease courses. AIDS Res Hum Retroviruses 2000;16:677-688.

178. Obert LA and Hoover EA. Relationship of lymphoid lesions to disease course in mucosal feline immunodeficiency virus type C infection. Vet Pathol 2000;37:386-401.

179. Ohyama H, Yamada T, Ohkawa A and Watanabe I. Radiation-induced formation of apoptotic bodies in rat thymus. Radiat Res 1985;101:123-130.

180. Okamoto Y, Douek DC, McFarland RD and Koup RA. Effects of exogenous interleukin-7 on human thymus function. Blood 2002;99:2851-2858.

181. Orandle MS, Crawford PC, Levy JK, et al. CD8+ thymic lymphocytes express reduced levels of CD8beta and increased interferon gamma in cats perinatally infected with the JSY3 molecular clone of feline immunodeficiency virus. AIDS Res Hum Retroviruses 2000;16:1559-1571.

182. Orandle MS, Papadi GP, Bubenik LJ, Dailey CI and Johnson CM. Selective thymocyte depletion and immunoglobulin coating in the thymus of cats infected with feline immunodeficiency virus. AIDS Res Hum Retroviruses 1997;13:611-620.

183. Ozben T. Oxidative stress and apoptosis: impact on cancer therapy. J Pharm Sci 2007;96:2181-2196.

184. Park E, Lee NH, Baik JS and Jee Y. Elaeocarpus sylvestris modulates gamma-ray-induced immunosuppression in mice: implications in radioprotection. Phytother Res 2008.

185. Pautler RG. Mouse MRI: concepts and applications in physiology. Physiology (Bethesda) 2004;19:168-175.

186. Pearse G. Histopathology of the thymus. Toxicol Pathol 2006;34:515-547.

187. Pedersen NC, Ho EW, Brown ML and Yamamoto JK. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science 1987;235:790-793.

188. Petrie HT. Cell migration and the control of post-natal T-cell lymphopoiesis in the thymus. Nat Rev Immunol 2003;3:859-866.

189. Petrie HT and Zuniga-Pflucker JC. Zoned out: functional mapping of stromal signaling microenvironments in the thymus. Annu Rev Immunol 2007;25:649-679.

190. Phillips JA, Brondstetter TI, English CA, Lee HE, Virts EL and Thoman ML. IL-7 gene therapy in aging restores early thymopoiesis without reversing involution. J Immunol 2004;173:4867-4874.

191. Pirronti T, Rinaldi P, Batocchi AP, Evoli A, Di Schino C and Marano P. Thymic lesions and myasthenia gravis. Diagnosis based on mediastinal imaging and pathological findings. Acta Radiol 2002;43:380-384.

192. Poli A, Pistello M, Carli MA, et al. Tumor necrosis factor-alpha and virus expression in the central nervous system of cats infected with feline immunodeficiency virus. J Neurovirol 1999;5:465-473.

193. Quarmby S, Kumar P and Kumar S. Radiation-induced normal tissue injury: role of adhesion molecules in leukocyte-endothelial cell interactions. Int J Cancer 1999;82:385-395.

194. Radtke F, Wilson A, Stark G, et al. Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity 1999;10:547-558.

195. Restrepo CS, Pandit M, Rojas IC, et al. Imaging findings of expansile lesions of the thymus. Curr Probl Diagn Radiol 2005;34:22-34.

196. Revest JM, Suniara RK, Kerr K, Owen JJ and Dickson C. Development of the thymus requires signaling through the fibroblast growth factor receptor R2-IIIb. J Immunol 2001;167:1954-1961.

197. Rich BE, Campos-Torres J, Tepper RI, Moreadith RW and Leder P. Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. J Exp Med 1993;177:305-316.

198. Rich BE and Leder P. Transgenic expression of interleukin 7 restores T cell populations in nude mice. J Exp Med 1995;181:1223-1228.

199. Rideout BA, Lowensteine LJ, Hutson CA, Moore PF and Pedersen NC. Characterization of morphologic changes and lymphocyte subset distribution in lymph

nodes from cats with naturally acquired feline immunodeficiency virus infection. Vet Pathol 1992;29:391-399.

200. Rijhsinghani A, Bhatia SK, Kantamneni L, Schlueter A and Waldschmidt TJ. Estrogen inhibits fetal thymocyte development in vitro. Am J Reprod Immunol 1997;37:384-390.

201. Rijhsinghani AG, Thompson K, Bhatia SK and Waldschmidt TJ. Estrogen blocks early T cell development in the thymus. Am J Reprod Immunol 1996;36:269-277.

202. Rodriguez-Galan MC, Bream JH, Farr A and Young HA. Synergistic effect of IL-2, IL-12, and IL-18 on thymocyte apoptosis and Th1/Th2 cytokine expression. J Immunol 2005;174:2796-2804.

203. Rossi FM, Corbel SY, Merzaban JS, et al. Recruitment of adult thymic progenitors is regulated by P-selectin and its ligand PSGL-1. Nat Immunol 2005;6:626-634.

204. Rossi SW, Jeker LT, Ueno T, et al. Keratinocyte growth factor (KGF) enhances postnatal T-cell development via enhancements in proliferation and function of thymic epithelial cells. Blood 2007;109:3803-3811.

205. Saito T and Watanabe N. Positive and negative thymocyte selection. Crit Rev Immunol 1998;18:359-370.

206. Sanchez-Cordon PJ, Romanini S, Salguero FJ, et al. Apoptosis of thymocytes related to cytokine expression in experimental classical swine fever. J Comp Pathol 2002;127:239-248.

207. Sands MJ and Levitin A. Basics of magnetic resonance imaging. Semin Vasc Surg 2004;17:66-82.

208. Sarli G, Della Salda L, Zaccaro L, Bendinelli M, Piedimonte G and Marcato PS. Apoptotic fraction in lymphoid tissue of FIV-infected SPF cats. Vet Immunol Immunopathol 1998;64:33-44.

209. Sasson SC, Zaunders JJ and Kelleher AD. The IL-7/IL-7 receptor axis: understanding its central role in T-cell homeostasis and the challenges facing its utilization as a novel therapy. Curr Drug Targets 2006;7:1571-1582.

210. Savino W. Neuroendocrine control of T cell development in mammals: role of growth hormone in modulating thymocyte migration. Exp Physiol 2007;92:813-817.

211. Savino W, Dalmau SR and Dealmeida VC. Role of extracellular matrix-mediated interactions in thymocyte migration. Dev Immunol 2000;7:279-291.

212. Savino W, Mendes-Da-Cruz DA, Smaniotto S, Silva-Monteiro E and Villa-Verde DM. Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix. J Leukoc Biol 2004;75:951-961.

213. Savino W, Postel-Vinay MC, Smaniotto S and Dardenne M. The thymus gland: a target organ for growth hormone. Scand J Immunol 2002;55:442-452.

214. Schluns KS, Kieper WC, Jameson SC and Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol 2000;1:426-432.

215. Schultz RD, Dunne HW and Heist CE. Ontogeny of the bovine immune response. Infect Immun 1973;7:981-991.

216. Scimone ML, Aifantis I, Apostolou I, von Boehmer H and von Andrian UH. A multistep adhesion cascade for lymphoid progenitor cell homing to the thymus. Proc Natl Acad Sci U S A 2006;103:7006-7011.

217. Sellon RK, Jordan HL, Kennedy-Stoskopf S, Tompkins MB and Tompkins WA. Feline immunodeficiency virus can be experimentally transmitted via milk during acute maternal infection. J Virol 1994;68:3380-3385.

218. Sempowski GD, Rhein ME, Scearce RM and Haynes BF. Leukemia inhibitory factor is a mediator of Escherichia coli lipopolysaccharide-induced acute thymic atrophy. Eur J Immunol 2002;32:3066-3070.

219. Shearer WT, Langston C, Lewis DE, et al. Early spontaneous abortions and fetal thymic abnormalities in maternal-to-fetal HIV infection. Acta Paediatr Suppl 1997;421:60-64.

220. Shelton GH and Linenberger ML. Hematologic abnormalities associated with retroviral infections in the cat. Semin Vet Med Surg (Small Anim) 1995;10:220-233.

221. Singh VK and Yadav VS. Role of cytokines and growth factors in radioprotection. Exp Mol Pathol 2005;78:156-169.

222. Smith CW. 3. Adhesion molecules and receptors. J Allergy Clin Immunol 2008;121:S375-379; quiz S414.

223. Srirajaskanthan R, Toubanakis C, Dusmet M and Caplin ME. A review of thymic tumours. Lung Cancer 2008;60:4-13.

224. Stebbing J, Gazzard B and Douek DC. Where does HIV live? N Engl J Med 2004;350:1872-1880.

225. Steinmann GG. Changes in the human thymus during aging. Curr Top Pathol 1986;75:43-88.

226. Sung YM, Lee KS, Kim BT, Choi JY, Shim YM and Yi CA. 18F-FDG PET/CT of thymic epithelial tumors: usefulness for distinguishing and staging tumor subgroups. J Nucl Med 2006;47:1628-1634.

227. Takahashi K, Inaoka T, Murakami N, et al. Characterization of the normal and hyperplastic thymus on chemical-shift MR imaging. AJR Am J Roentgenol 2003;180:1265-1269.

228. Tateishi Y, Sasabe E, Ueta E and Yamamoto T. Ionizing irradiation induces apoptotic damage of salivary gland acinar cells via NADPH oxidase 1-dependent superoxide generation. Biochem Biophys Res Commun 2008;366:301-307.

229. Tedder TF, Steeber DA, Chen A and Engel P. The selectins: vascular adhesion molecules. Faseb J 1995;9:866-873.

230. Tompkins MB, Bull ME, Dow JL, et al. Feline immunodeficiency virus infection is characterized by B7+CTLA4+ T cell apoptosis. J Infect Dis 2002;185:1077-1093.

231. Tompkins MB and Tompkins WA. Lentivirus-induced immune dysregulation. Vet Immunol Immunopathol 2008.

232. Torten M, Franchini M, Barlough JE, et al. Progressive immune dysfunction in cats experimentally infected with feline immunodeficiency virus. J Virol 1991;65:2225-2230.

233. Troyer JL, Vandewoude S, Pecon-Slattery J, et al. FIV cross-species transmission: An evolutionary prospective. Vet Immunol Immunopathol 2008;123:159-166.

234. Urdal DL and Park LS. Studies on hematopoietic growth factor receptors using human recombinant IL-3, GM-CSF, G-CSF, M-CSF, IL-1 and IL-4. Behring Inst Mitt 1988:27-39.

235. Utsuyama M and Hirokawa K. Age-related changes of splenic T cells in mice--a flow cytometric analysis. Mech Ageing Dev 1987;40:89-102.

236. Utsuyama M and Hirokawa K. Hypertrophy of the thymus and restoration of immune functions in mice and rats by gonadectomy. Mech Ageing Dev 1989;47:175-185.

237. van Baarlen J, Schuurman HJ and Huber J. Acute thymus involution in infancy and childhood: a reliable marker for duration of acute illness. Hum Pathol 1988;19:1155-1160.

238. Van Herle AJ and Chopra IJ. Thymic hyperplasia in Graves' disease. J Clin Endocrinol Metab 1971;32:140-146.

239. Van Oers NS, Garvin AM, Davis CB, et al. Disruption of CD8-dependent negative and positive selection of thymocytes is correlated with a decreased association between CD8 and the protein tyrosine kinase, p56lck. Eur J Immunol 1992;22:735-743.

240. Vanlangenakker N, Berghe TV, Krysko DV, Festjens N and Vandenabeele P. Molecular mechanisms and pathophysiology of necrotic cell death. Curr Mol Med 2008;8:207-220.

241. von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE and Murray R. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med 1995;181:1519-1526.

242. Waldmann TA, Dubois S and Tagaya Y. Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. Immunity 2001;14:105-110.

243. Wang Y, Tokuda N, Tamechika M, et al. Vascular and stromal changes in irradiated and recovering rat thymus. Histol Histopathol 1999;14:791-796.

244. Waselenko JK, MacVittie TJ, Blakely WF, et al. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Ann Intern Med 2004;140:1037-1051.

245. Weaver CC, Burgess SC, Nelson PD, et al. Placental immunopathology and pregnancy failure in the FIV-infected cat. Placenta 2005;26:138-147.

246. Weaver CT, Hatton RD, Mangan PR and Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25:821-852.

247. Weetman AP. The immunology of pregnancy. Thyroid 1999;9:643-646.

248. Whalen BJ, Marounek J, Mordes JP, Rossini AA and Greiner DL. Type 1 cytokines polarize thymocytes during T cell development in adult thymus organ cultures. J Autoimmun 2003;20:27-42.

249. Wilfert CM and Fleming T. Perinatal transmission--successful interventions. Where do we go from here? Int J STD AIDS 1998;9 Suppl 1:22-27.

250. Wilkinson PC and Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. J Exp Med 1995;181:1255-1259.

251. Willett BJ, McMonagle EL, Logan N, Spiller OB, Schneider P and Hosie MJ. Probing the interaction between feline immunodeficiency virus and CD134 by using the novel monoclonal antibody 7D6 and the CD134 (Ox40) ligand. J Virol 2007;81:9665-9679.

252. Wittram C, Fischman AJ, Mark E, Ko J and Shepard JA. Thymic enlargement and FDG uptake in three patients: CT and FDG positron emission tomography correlated with pathology. AJR Am J Roentgenol 2003;180:519-522.

253. Woo JC, Dean GA, Pedersen NC and Moore PF. Immunopathologic changes in the thymus during the acute stage of experimentally induced feline immunodeficiency virus infection in juvenile cats. J Virol 1997;71:8632-8641.

254. Wu L, Scollay R, Egerton M, Pearse M, Spangrude GJ and Shortman K. CD4 expressed on earliest T-lineage precursor cells in the adult murine thymus. Nature 1991;349:71-74.

255. Wykrzykowska JJ, Rosenzweig M, Veazey RS, et al. Early regeneration of thymic progenitors in rhesus macaques infected with simian immunodeficiency virus. J Exp Med 1998;187:1767-1778.

256. Yamamoto JK, Pedersen NC, Ho EW, Okuda T and Theilen GH. Feline immunodeficiency syndrome--a comparison between feline T-lymphotropic lentivirus and feline leukemia virus. Leukemia 1988;2:204S-215S.

257. Yamamoto JK, Pu R, Sato E and Hohdatsu T. Feline immunodeficiency virus pathogenesis and development of a dual-subtype feline-immunodeficiency-virus vaccine. Aids 2007;21:547-563.

258. Yamamoto JK, Sparger E, Ho EW, et al. Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. Am J Vet Res 1988;49:1246-1258.

259. Yamanaka K, Nakayama H, Watanabe K and Kameda Y. Anterior mediastinal mass in a patient with Graves' disease. Ann Thorac Surg 2006;81:1904-1906.

260. Yarilin AA and Belyakov IM. Cytokines in the thymus: production and biological effects. Curr Med Chem 2004;11:447-464.

261. Ziegler SF and Liu YJ. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. Nat Immunol 2006;7:709-714.

262. Zubkova I, Mostowski H and Zaitseva M. Up-regulation of IL-7, stromal-derived factor-1 alpha, thymus-expressed chemokine, and secondary lymphoid tissue chemokine gene expression in the stromal cells in response to thymocyte depletion: implication for thymus reconstitution. J Immunol 2005;175:2321-2330.