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Histopathological Changes in the Thymus of Mouse After Radiation Exposure

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ABSTRACT: The thymus is a primary lymphoid organ that manifests dynamic physiological changes as mice age in addition to being exquisitely sensitive to stress and toxic insult. It is typically the first lymphoid tissue to respond to immunotoxic xenobiotics, with the first change being loss of cortical lymphocytes by apoptosis. This is followed by removal of the apoptotic cellular debris and, in the absence of recovery, may lead to loss of the corticomedullary demarcation and organ atrophy. Nonneoplastic proliferative changes include focal lymphoid hyperplasia and proliferation of medullary epithelial cells, often with formation of ribbons, cords, or tubules. Thymomas are relatively rare tumors that exhibit a wide spectrum of morphologic types but do not metastasize. Thymic lymphomas are common in some mouse strains and can become leukemic with hematogenous spread throughout the body after radiation exposure.

KEYWORDS: thymus, histopathological, mouse, radiation, hyperplasia, toxic, morphologic, atrophy

I.INTRODUCTION

The immune status of the thymus, as reflected in the histological appearance, and/or changes in the relative organ weight, varies according to factors such as the age and genetic background, the adequacy of nutrition, the stress levels, and hormonal status of mouse in addition to, and potentially interfering with, the interpretation of exposure to xenobiotics.¹ According to Kuper: "The primary limitation of histopathology is that a tissue section represents a static time point in a dynamic process. Therefore, the dynamics of the immune system should be carefully considered in the histopathologic assessment of immunotoxicity" In addition to the thymus not being a static histologic entity, the interchangeable use of interpretive and descriptive terms has led to overlapping terminology for the same morphological change, potentially causing confusion and poor communication. For example, when there is a decrease in thymic size and cellularity, it is always appropriate to use descriptive terminology such as "reduced numbers of cortical lymphocytes" and "increased numbers of tingible body macrophages." However, similar or even identical changes may be diagnosed as "atrophy" or "involution," especially in the evaluation of tissues from older mice in chronic studies. Normal development, histology, and function of the thymus have been reported previously². The use of standardized descriptive nomenclature with respect to thymic pathology is addressed elsewhere This paper focuses on morphological features of nonproliferative and proliferative lesions of the rodent thymus by radiation exposure.³ A variety of factors and conditions result in an alteration in the cellular density and cellular composition of the thymus. Most commonly recognized is a decrease in lymphocytes resulting from a range of background physiological influences and the immunosuppressive effect of xenobiotics and radiation exposure.⁴

Normal age-associated decreases in cellularity are termed involution, whereas induced reductions such as from inadequate nutrition, stress, or toxicity represent thymic atrophy. The histological appearance of the thymus under these varied conditions is similar, since the end point is the reduction in cortical lymphocytes, and shrinkage of the thymic lobules. Increased numbers of apoptotic bodies and tingible body macrophages are followed by lymphocyte depletion and increased prominence of interlobular septae and eventually an inverse of the normal histological picture, where the medulla has a higher cellular density than the cortex. Factors such as stress and toxicity can simultaneously be superimposed on the normal ageing process of lymphocyte reduction. Consequently, the distinction between atrophy

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and involution in older mice can be problematic. In such situations, the best course of action is to (1) assess changes in cell density and compartment size, (2) take into consideration comparison with concurrent untreated controls, (3) factor in the temporal sequence of events, (4) dose-response findings, (5) and the totality of clinical findings including histological changes in other tissue and from there deduce the appropriateness of interpreting the change atrophy versus involution.⁵

The age of the mouse during radiation exposure plays a large part in the level of cellularity of the thymus and its overall histological appearance. Physiological involution reflects the change in function of the thymus from lymphocyte production to recirculation. In the intact rodent, involution is a normal, gradual, and, irreversible aging change that begins at puberty and is considered to be associated with increased circulating levels of sex steroids. Gonadectomy will delay involution in both sexes showed that orchidectomy restored the thymus and raised the total white cell count in 18-month-old mice in which the thymus had virtually disappeared. Similar thymic regeneration was achieved in intact old mice with subcutaneous implants of lutenizing hormone releasing hormone (LHRH). Histologically, involution is characterized by a reduction in the size of the thymus with a decrease in cortical lymphocytes, thinning and irregularity of the cortex, and loss of corticomedullary demarcation. At the corticomedullary junction, there is an increase in perivascular spaces, and perivascular B lymphocyte and plasma cell populations, which may form lymphofollicular structures with prominent germinal centers. There is infiltration by adipose tissue in the connective tissue capsule and septae by radiation exposure. In the medulla, epithelial cells become progressively more prominent. The microscopic changes in epithelial cells that accompany involution can show considerable pleomorphic variation in distribution, architectural arrangement, and cytological appearance. Undoubtedly, there is some degree of cell proliferation as the epithelial cells are sometimes arranged in cords or ribbons and may form tubules or cysts lined by cuboidal to squamous epithelium. Epithelial changes are more prominent in females than in male mice.⁶

Species, strain, and sex differences occur in the evolution of age-dependent thymic changes. Complete involution typically does not occur in any species, including humans; there is usually some stroma with remnants almost entirely composed of epithelial cords and tubules. Changes are much less pronounced in the mouse when compared with the rat. In mice, thymic cyst formation due to radiation becomes more prominent with age and specific radiation doses like alpha, beta or gamma rays, infrared rays or uv rays. Strain differences can be significant. For example, the thymus of mice exposed with infrared rays consisted mostly of epithelial cords or tubules with few lymphocytes, whereas epithelial components are more scarce in aged females of the non-exposed mice. In ageing female exposed by uv rays the cellular depletion of the cortex is accompanied by follicular expansion of small lymphocytes comprising a subset of sex-dependent mature T cells, and the emergence of B cells and plasma cells in the medulla.⁷

General undernutrition, and specific deficiencies of Vitamin B6, amino acids, fatty acids, and minerals such as zinc cause immunosuppression and a decrease in thymic weight occurred due to exposure by gamma rays. In addition, feed and/or water restriction are significant stressors, resulting in secondary immunosuppression via elevated adrenocortical hormone levels occurred by exposure due to alpha radiations.⁸

Stress causes elevated circulating levels of glucocorticosteroids mediated by the hypothalamus-pituitary-adrenal axis. The thymus is the most sensitive of the lymphoid tissues to changes in adrenocortical hormone levels and a decrease in thymic weight occurs due to loss of cortical lymphocytes by beta radiation exposure. The splenic white pulp and lymph nodes can be similarly affected, although to a lesser degree. The initial response of apoptosis of cortical lymphocytes can be seen within hours of treatment with the synthetic glucocorticoid dexamethasone, followed by removal of apoptotic debris by macrophages as seen due to high radiation dosages. Such changes due to acute debilitating disease are common and may be prominent in mice sacrificed in a moribund condition. Known environmental stressors are social ranking within gang-housing systems, immobilization, as well as excessive changes in temperature or humidity and restriction of access to food and water as well as different kinds of radiations. These changes are usually reversible on removal of the stressor.⁹

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II.DISCUSSION

The thymus is the lymphoid organ that shows the largest response to hormonal fluctuations. Increased levels of sex steroids have a profound effect on the thymus, ultimately resulting in involution, commencing at the onset of puberty. Studies in mice have shown that cells in the thymus staining with CD8 α (Lyt-2) monoclonal antibody are particularly sensitive to sex hormone action by radiation it occurs fast. Generally, estrogen reduces and androgens maintain CD8 α (Lyt-2) cells due to alpha rays. In pregnant females, an early increase in thymic weight is followed by a marked reduction in cellularity of the cortex. The level of cellularity returns to normal once pregnancy is over in low radiation doses. Increased levels of progesterone during pregnancy have a negative effect on thymic weight, whereas increased prolactin occurring during lactation has a stimulatory effect on the thymus. Thyroxine and growth hormone also have a stimulatory effect and decreased levels of growth hormone have been associated with a reduction in thymic weight.¹⁰ Immunotoxicity refers to the potentially harmful effects that physical, chemical, or other agents have on the immune system like chemical bleaching agents, acids, bases, alcohols etc. The dynamics of the immune system, with its ongoing cellular proliferation and differentiation, lymphocyte trafficking, and gene amplification, make it highly susceptible to toxic insults particularly in the thymus and bone marrow where rapid cell turnover occurs. Since generation of T cells by the thymus is particularly important early in life, immunotoxicants may show their effects in particular, and in lower doses, during the prenatal and the early postnatal period if high radiation dosages are given.¹¹ The consequences of immune dysfunction are immunosuppression or immunoenhancement, and either type of immunomodulation can provoke hypersensitivity or autoimmunity. Immunotoxic reactions manifest themselves most commonly as immunosuppression characterized by selective or generalized depression of the lymphoid organs. However, there may be dramatic species differences in response to given immunotoxicants at different radiation dosages.¹²

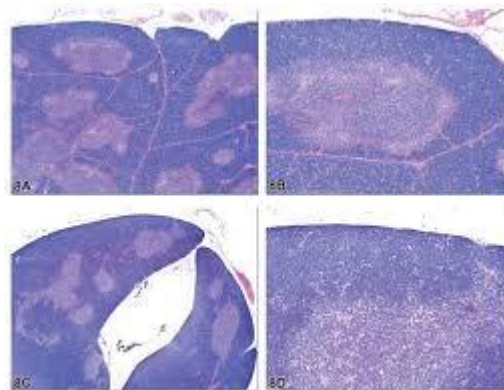


Fig.1 .Histological changes in thymus of mouse by different doses of radiation

The most sensitive indicator of immunosuppression, particularly in short-term studies, is a decrease in relative thymus weight, which may or may not have a corresponding detectible morphological finding of decreased cellularity in the cortex, and less commonly the medulla or both. However, high doses of an immunotoxicant can cause overt toxicity or a decrease in food and/or water consumption and severe stress resulting in nonspecific (secondary) inhibitory effects on the immune system. A decrease in thymus:body weight ratio, therefore, cannot be used as stand-alone criterion of immunosuppression. In some cases a dose-response relationship as well as changes in other lymphoid tissues may be of some help in deciding whether thymic atrophy is a direct effect of immunosuppression or nonspecific response to stress specifically which occurred due to infra red dosages.¹³

The thymus is especially sensitive to exposure to immune system toxicants, and often there is a clear dose-associated decrease in size of the thymus secondary to apoptosis of cortical lymphocytes. The histological changes present are

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dependent upon the dose of the immunotoxicant and when, in the dynamic process, the thymus is examined during radiation exposure. Following apoptosis of cortical lymphocytes and their removal by macrophages, a decrease in cortical cellularity, characterized by thinning and loss of the cortex and blurring of normal corticomedullary demarcation are seen. At a sufficiently high dose of an immunotoxicant, there may be degeneration of epithelial cells and epithelial cell proliferation with development of glandular structures containing eosinophilic material due to high dosages of uv rays.¹⁴

The immune system has a high regenerative capacity, and, depending upon the degree of toxicity, can recover in a relatively short period of time following a toxic insult. Therefore the time between chemical insult and analysis is also an important consideration in immunotoxicity testing which is directly proportionate to dosage of radiation.¹⁵ For interpretation of immunopathology, it is important to remember that the thymus is not a fixed histological entity. The level of cellularity will vary according to a number of background factors. Not all effects on the thymus seen in regulatory studies are due to the immunomodulatory effects of the test material and the use of age-matched control mice is critical. To assist with the evaluation of thymic weight data, it is recommended that an historical database should be developed and maintained for each species and strain used and should include the age, weight and sex of mouse which is given radiation.¹⁶

III.RESULTS

Other non-proliferative lesions are uncommon. Occasional developmental anomalies resulting in ectopic thyroid, parathyroid, or thymic tissue as well as intrathyroid cysts have been reported due to alpha, beta, gamma, IR and uv rays. Inflammatory and vascular lesions are rare in the rodent thymus. Primary inflammation by extension from adjacent tissues is always possible. Multifocal dystrophic mineralization secondary to renal or parathyroid disease may be observed. Hemorrhage and fibrosis have been seen occasionally with the former, possibly a consequence of esophageal perforation during gavage administration of radiations.¹⁷

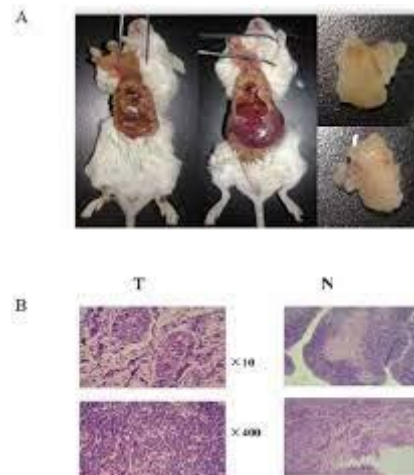


Fig.2. Thymic lymphoma due to uv radiations in dissected mouse

Epithelial hyperplasia may be present as an age-associated lesion with relatively high incidence in some strains of rodent. It may be focal or diffuse and is particularly common in mice, occurring more frequently and with a higher secretory activity in females. Component cells are cuboidal to columnar, often form tubules or cords, are occasionally ciliated, and may have an admixture of secretory (goblet) cells. Tubules may contain variable amounts of eosinophilic secretory material. Unusual cellular forms may be seen. Since epithelial hyperplasia is seen in greater frequency as mice age, it is often present in thymuses that are undergoing involution. Furthermore, the pleomorphic variations of

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thymic epithelial hyperplasia are similar to the spectrum of cellular forms of thymoma; consequently, extensive hyperplastic lesions may be difficult to distinguish from early benign thymomas. Treatment-related epithelial hyperplasia has been seen with diethylstilbesterol administration in mice. Epithelial cyst formation has been recorded in mice treated with exogenous estrogen in addition to gamma rays.¹⁸

Lymphoid hyperplasia occurs in older rodents, especially mice greater than 6 months old. It is more common in females, may be unilateral or bilateral and focal or diffuse . It consists of proliferations of pleomorphic lymphoid cells, often near the corticomedullary junction. When focal it is typically well demarcated and may resemble a lymphoid follicle. Lymphoid follicle development has been reported in female NZB × SJL hybrid mice. Epithelial and lymphoid hyperplasia may occur together and, because of its prevalence in older rodents, it may be seen in thymuses undergoing involution by radiation dosages getting higher.¹⁹

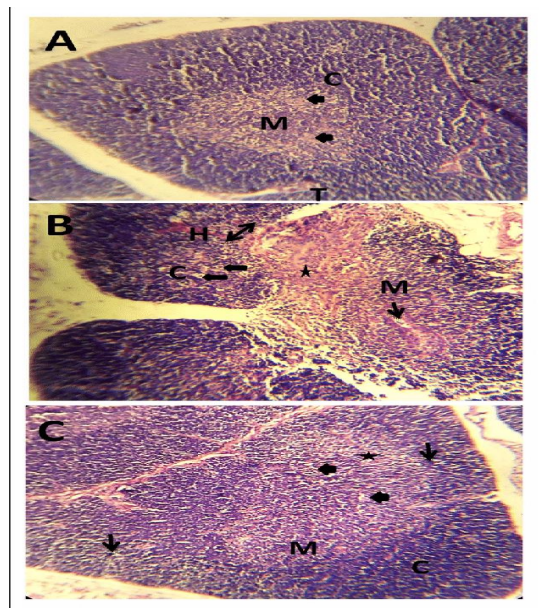


Fig.3. Gamma radiation induced histological changes in mouse

The neoplasm of thymic epithelial cells is characterized by a variable admixed population of lymphoid cells .Neoplastic epithelial cells may be localized or dispersed among lymphoid cells .It is likely that the gradation between epithelial hyperplasia and thymoma may present diagnostic challenges, especially for the more diffuse proliferative lesions. While it has been reported that thymomas in rodents can be divided into 3 main histological groups, depending on the relative preponderance of epithelial and lymphoid cells occurred according to radiation types.²⁰

The neoplastic epithelial cells in thymomas can vary considerably in their arrangement and appearance. Thymomas may be epidermoid forming nodules of nonkeratinizing squamous epithelium; consist of squamoid cells with some areas of keratinization , form papillary lesions with cystic areas ,form ribbons, cords or tubules and can occur as spindleoid cells . Some less common types include thymomas with endocrine (adenoid) growth patterns ,a neuroendocrine phenotype and a myoid thymoma with skeletal muscle differentiation due to high level of infrared radiations.²¹

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IV. CONCLUSIONS

Neoplasms of the thymus of mice are most commonly T-cell lymphomas of thymic lymphocyte origin. T-cell lymphoma can occur spontaneously in young mice <3 months of age. The mice spontaneously occurring thymic lymphomas are rare but can readily be induced by chemicals, viruses, irradiation, and in some types of tumor suppressor gene knockout mice. Most spontaneous and induced lymphoblastic lymphomas in the mouse arise unilaterally in the thymus. The initial gross lesion is a reduction in thymic size (thymic atrophy).²² Cortical atrophy is characterized by loss of cortical lymphocytes with thinning of the cortex. There is maintenance of normal lobular architecture. Subsequent to cortical atrophy, a preneoplastic stage of thymic lymphoma called atypical hyperplasia has been described in chemically treated and radiated mice. Atypical hyperplasia may be unilateral or bilateral. There is a diffuse change with loss of the normal corticomedullary demarcation. Normal architecture is replaced by sheets of large, atypical lymphocytes and fewer admixed small lymphocytes. This preneoplastic lesion can be differentiated from lymphoma by the heterogeneous cell population, variable mitotic index, and failure of lymphocytes to extend beyond the capsule of the thymus.²³

Ultimately, there is enlargement of the affected thymus lobe by nodular lymphocytic proliferation which progresses to generalized involvement of the thymus and mediastinum and finally dissemination in the blood with multiorgan involvement by radiations. Histologically, there is obliteration of the normal thymic architecture by broad sheets of a uniform population of lymphoblasts, which may extend through the thymic capsule with infiltration into surrounding tissues. Component cells are large, with moderate amounts of cytoplasm, vesicular, sometimes irregular nuclei and prominent central nucleoli. Mitoses are common due to types of radiations given.²³

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