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Dose Dependence of the Severity of Radiation-Induced Thymic Lymphoma in Mice

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ABSTRACT : **The dose dependence of the severity of radiation-induced thymic lymphoma in C57BL/6J mice was studied. Mice were exposed to fractionated irradiation at the total doses of 4.0, 6.0 and 8.0 Gy (four irradiations at 8-day intervals) starting from 33 days after birth. Pathological and histological changes of each mouse were observed after periodical sacrifice at day 75, 100, 125, 150, 175, 200, 250, 300 after the first irradiation. The severity of cancers were classified into 4 stages by clinical signs with respect to the enlargement of the thymus, spleen, liver, the progression of the cancer in the thymus, and the metastasis to the spleen, liver, lung and the lymphatic nodes. Among the 490 mice observed, 146 mice had thymic lymphoma. A clear dose-effect relationship was observed as well as the dose-response relationship. Also, periodical observation showed that thymic lymphoma was first induced in mice sacrificed at day 100 (130days old), and metastasize in the order of spleen, lung, liver and then the lymphatic nodes. The results suggest that radiation may be involved not only as a tumor initiator but also as a tumor promoter, and a tumor progression-enhancing agent.**

Keywords : **dose-effect relationship, radiation-induced thymic lymphoma, cancer severity, serial observation**

Introduction

Stochastic effect, unlike deterministic effect, has been defined as one in which the probability of the effect occurring increases continuously with increasing dose while the severity of the effect is independent of the magnitude of the dose (ICRP *et al.*). But, there has been some researches pointing out that more severe skin cancers (squamous cell carcinoma) were observed in subjects who were exposed to higher doses (Kaplan *et al.*, 1950; Kaplan *et al.*, 1952; Kaplan *et al.*, 1964). To our knowledge, no animal experimental data are available on this assumption concerning radiation-induced cancer. In re-examining this assumption, the ordinary life-long observing method used in most radiation-induced cancer researches would not be suitable for evaluating the severity of cancer (Kaplan 1967; Kusama 1982). By this method, observation of the animals would be at the point of its death, hence meaning that the cancer observed be always fatal. In this study, the dose dependence of the severity of radiation- induced thymic lymphoma was re-examined experimentally using C57BL/6J mice. In order to classify the severity, and observe cancer growth in serial motion in detail, mice were sacrificed on the periodical day planned in advance, and the conditions of tumor progressions were observed pathologically and histologically. This observing method has proven to be an excellent tool to get information of the starting point, the state of progression, and the way of metastasis of radiation- induced thymic lymphoma.

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Materials and Methods

Irradiation

C57BL/6J mice were bought from Charles River Japan Co., and kept under standard laboratory conditions; temperature 22±2Å, air humidity 60-65%, light control 10A.M.-10P.M. Food and water were provided ad libitum. 9-18 weeks old female mice were mated with 9-18 weeks old male mice from 6P.M.-10A.M., and the females were examined for vaginal plugs at 10A.M. the next day. The F1 mice born from these females were weaned at day 33, and used for irradiation experiments. Mice were exposed on whole body to fractionated irradiation at the total doses of 4.0, 6.0 and 8.0Gy (four irradiations at 8-day intervals) starting from 33days after

birth. In this way, thymic lymphomas can be enhanced in 5 to 6 months in 90% of the irradiated mice (#ref. Kaplan *et al.*). Single dose exposed were 1.0, 1.5, and 2.0 Gy with dose rate of 0.35 Gy/min. performed by the X-ray (225 kV) at Suzuka University of Medical Science. After irradiation, each mouse was checked twice a day of health conditions.

The non-irradiated group was also weaned at day 33 and kept as a control group.

Pathological Observation and Histology

Each mouse was sacrificed on day 75, 100, 125, 150, 175, 200, 250, 300 after the first irradiation, and induction of thymic lymphoma and other tumors such as lymphatic leukemia, myeloid leukemia, etc. was checked. Then, sections of the thymus, lung, liver, spleen, kidney, and parts of tumor tissues were taken from each mouse for histological examination. The specimens for histological examinations were prepared by Bouin fixation and embedded in paraffin. The staining was done by conventional Hematoxilin-Eosin stain. For histopathological diagnosis we used WHO's Pathology of Tumors in Laboratory Animals as a sample guide. The types of tumors classified in this study are shown in Table 1.

Severity Classification

First, the degree of tumor progression and metastasis was observed for subjects that had thymic lymphoma. The organs for histological inspection were the thymus, spleen, lung, and liver. The degree of tumor progression and metastasis was classified into 5 stages, 0 to stage 4, in which stage 0 stands for no metastasis. The degrees of metastasis to the lymphatic nodes were also examined. For the lymphatic nodes the inspection was done pathologically and the system of categorization is shown in Table 2. Then, the severity of thymic lymphoma was classified putting together the results: the degree of tumor progression in the thymus, the degree of metastasis to the spleen, lung and liver, the degree of metastasis to the lymphatic nodes. The severity was classified into 4ranks; severity 1 to severity 4, in which severity 4 is the most severe thymic lymphoma. The categorization of the severity is shown in Table 3.

Results

Hazard rate and dosage reaction relation of a thymus lymphoma

Among 103 control mice, we judged 94 of them that

Table 1. Tumor classification of mice

Lymphoma	Thymus Lymphoma
	Non-Thymus Lymphoma
Leukemia	Lymphocyte Leukemia
	Myeloid Leukemia
Others tumors	Ovary tumor, Lung tumor, Hepatoma, Harderian tumor, Breast tumor, Salivary Gland tumor, Others

Tablre 2. Experimental group and the number of use animal (♂ / ♀).

Observation day Irradiation groups	75	100	125	150	175	200	250
0	9/12	10/10	-	10/12(2)	-	10/10	10/10
1.0Gy×4 times	-	11/10	-	11(1)/11	-	10/12(2)	10(1)/11(2)
1.5Gy×4 times	9/11	10/12(2)	12(3)/12(3)	10/15(7)	11(2)/8(4)	9/6	-
2.0Gy×4 times	8/11	10/10	14(5)/14(6)	14(5)/22(14)	11(4)/2(2)	2/4(2)	-

*All the observation days are the days since first time irradiation day.

**Numerical value in a parenthesis is number of the mice that died before a plan observation day and the mice, which we butcher it just before death because I weakened and observed.

Table 3. Judgment basis of degree of seriousness of a thymus lymphoma by pathologic anatomy

Degree of severity	Judgment basis
1	Weight only for a thymus increases
2	Hypertrophy of a thymus and slight enlargement of spleen are recognized
3	A thymus, hypertrophy of spleen and slight enlargement of liver are recognized
4	Remarkable hypertrophy is recognized by thymus, spleen, liver

a thymus lymphoma judged not to occur from of general condition, pathology and a histological observation result with tumor-free mice. Mice diagnosed as a thymus lymphoma as a result of observation of all general condition out of 436 mice, the pathology that I observed by this study and histology is 144. Because it was clear that there was not a difference, as a result of being equal to or less than it, I did it with treating mice of male and female in a mass in hazard rate of a thymus lymphoma of male and female.

We show hazard rate and dosage of accumulation hazard rate of a thymus lymphoma, alteration according to observation day in each Fig. 1, Fig. 2. In addition, because there is not a group in 0 Gy, 1.0 Gy in Fig. 2 for group, 175 days for 125 days, we can seem to compare it with other bombardment groups directly, and I take advantage of data of a group in factor between time for group, 250 days for group, 200 days for 150 days, and it is done a plot. The thymus lymphoma was recognized in 2/21 mice of them, 2.0 Gy in 0 Gy in a group on 75th by 1/19 of them. For this, outbreak of a thymus lymphoma is recognized each dosage group in a group together for 100 days. As for the time of outbreak of a thymus lymphoma, a certain thing was suggested without relation in dosage during 100 days since 75th since first time bombardment day by this thing. Hazard rate of 1.0 Gy does 1.5 Gy, 2.0 Gy and rate in a group for 100 days, and, in Fig. 1, what is high was recognized, but this is because there is a little incidence of mice (p=0.0033). Dosage reaction reference is recognized in a group for group, 175 days for 150 days by hazard coefficient of a thymus lymphoma that I try to do comparison between dosages in each observation day group. In 2.0 Gy for

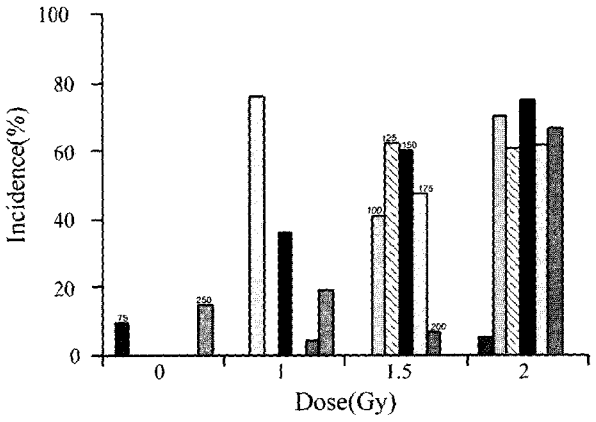

Fig. 1. Incidences of thymic lymphoma.

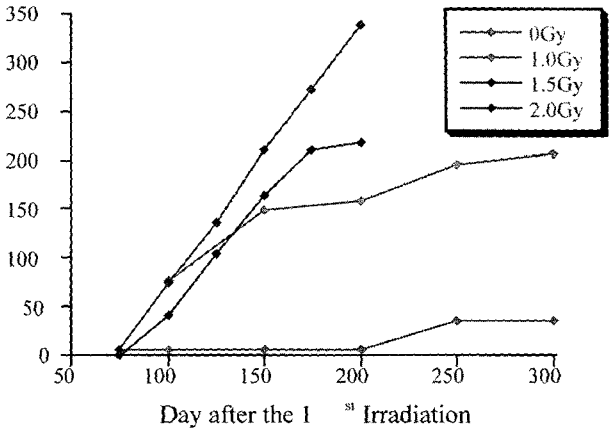

Fig. 2. Cumulative incidences of thymic lymphoma in C57BL/6J Mice.

what increase determination relaxes in a group for group, 175 days for 1.0 Gy, 1.5 Gy are monotonous to a group 150 days for 200 days, and increase is clear when I watch it by accumulation hazard rate of a thymus lymphoma of Fig. 2.

Base data to judge degree of seriousness of a thymus lymphoma

Thymus weight of tumor-free mice; Increase of thymus weight is one of an important standard of diagnosis basis of a thymus lymphoma. Weight of a thymus decreases with day instars of mice. Thus I show thymus weight of the C57BL/6J tumor-free mice that I used by this study in Fig. 3 in day by groups because I have to grasp thymus weight of tumor-free mice in course days.

In a 75th group of tumor-free mice, mean of thymus

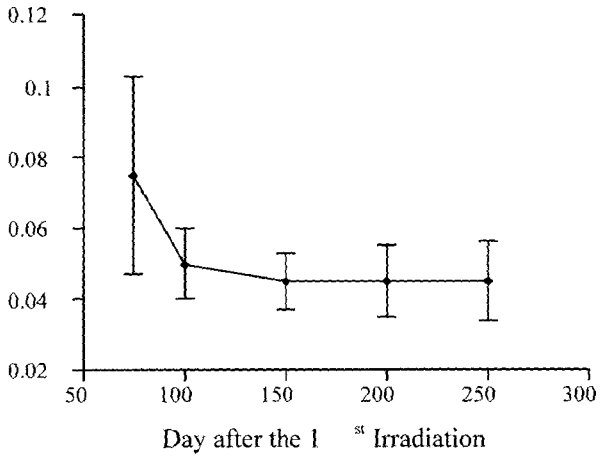

Fig. 3. Mass of thymus in normal mice.

we.ght and standard deviation are each 0.075 g, 0.028 g and a variation index (cv) makes rate other groups, and individual difference compares it with other stage greatly and understands a big thing. As for the thymus weight, a dif'erence by day instars is not recognized after a group for 150 days and it is it with 0.045±0.01 g without relation in weight of a mouse individual.

A change of the course days of thymus weight of thymus lymphoma laboratory mouse

The mouse which exceeded this as the weight upper limit va.ue of a normal thymus in double of σ of an every da.y group on the basis of course days of tumor-free mice thymus weight of a thymus lymphoma judged it that might de'elop it, and did diagnosis by diagnosis of histopathology, and confirmed presence of a thymus lymphoma. I show thymus weight mean of thymus lymphoma laboratory mouse in Figure 4 in day by groups according to dosage.

There is not a change of day instars of thymus weight in control group of thymus lymphoma laboratory mouse, bu: increase of thymus weight is recognized with day instars in bombardment group. Besides, I am remarkable increase of thymus weight high laboratory mouse of dosage in both day groups.

A change of the course days of spleen weight of tumor-free laboratory mouse

We show spleen weight mean of the tumor-free mouse that we observed in Figure 5 in day by groups. Relation with the day instars that seems to have been noted in a thymus is not recognized in spleen. Spleen weight mean of the whole tumor-free mice is 0.072±0.012 g. As for the spleen weight, correlation with mice weight was not recognized, too.

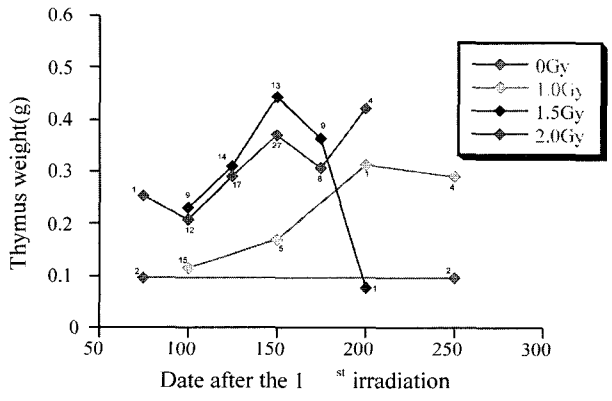

Fig. 4. Mass of thymus in mice baring thymic lymphoma.

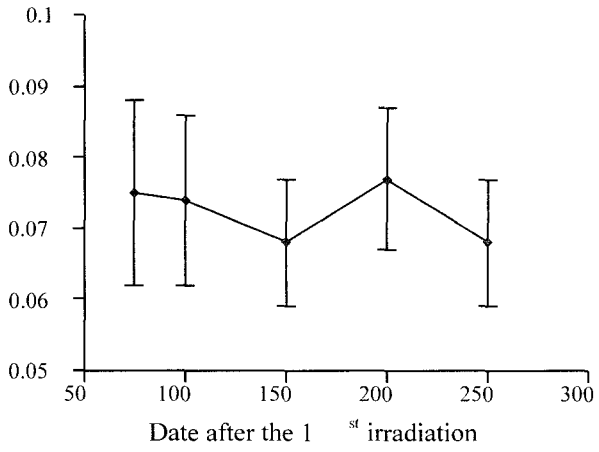

Fig. 5. Mass if spleen in normal mice.

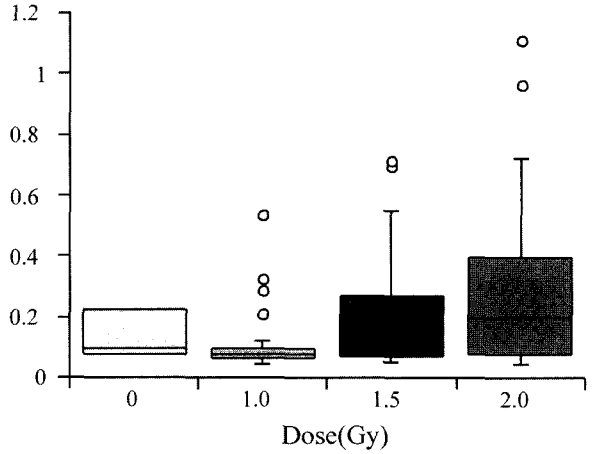

Fig. 6. Mass of spleen in mice baring thymic lymphoma.

Spleen weight of tumor-free mice gathers up all observation day instars mice about spleen from a difference of day instars not being recognized and shows the thing which did a box beard plot according to dosage in Figure 6. Spleen weight of thymus lymphoma laboratory mouse is distributed over 0.039-1.109 g and understands that I turn rate into tumor-free laboratory mouse and am enlarged clearly. A range of distribution of spleen weight became wide, and dosage dependence was recognized with increase of dosage, too.

A change of the course days of liver weight of tumor-free laboratory mouse

I show liver weight of tumor-free mice in Figure 7 in day by groups.

As day instars increases, liver weight increases. However, I show the consequence that a plot did reference with

mice weight and liver weight because day instars adds to this, and weight increases in Figure 8. Between weight and liver weight of laboratory mouse, meaningful correlation (coefficient of correlation R=0.86) is recognized. We show the thing that did a plot of liver weight of thymus lymphoma laboratory mouse by a box beard graph according to dosage in Figure 9.

With increase of dosage, mean of liver weight increases, and in particular liver weight of 2.0 Gy group is high conspicuously. In addition, as for the distribution of liver weight, it is admitted that a range of distribution becomes wide with increase of dosage by 0.719-5.836 g.

An organization diagnosis results

From a thymus of thymus lymphoma laboratory mouse, spleen, liver, observation consequence of histology of

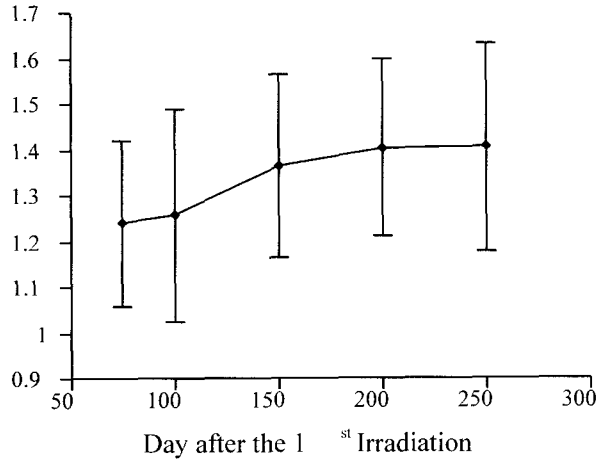

Fig. 7. Mass of liver in normal mice.

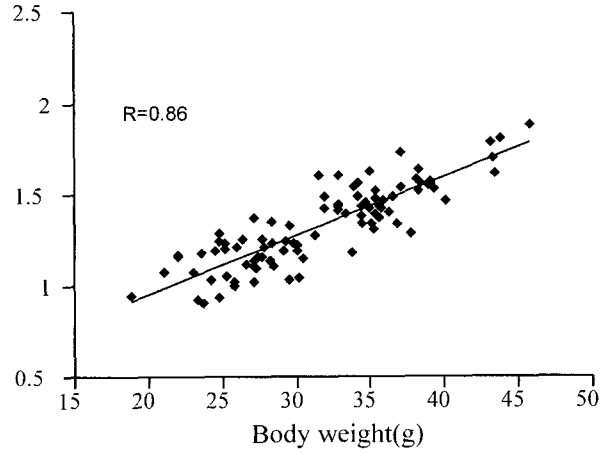

Fig. 8. Relationship between liver weight and body weight in normal mice.

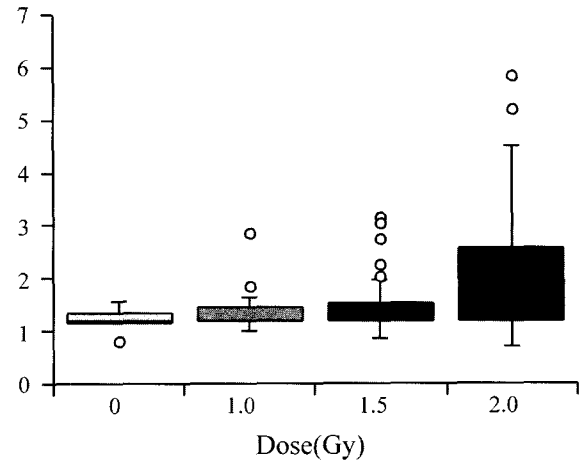

Fig. 9. Mass of liver in mice bearing thymic lymphoma.

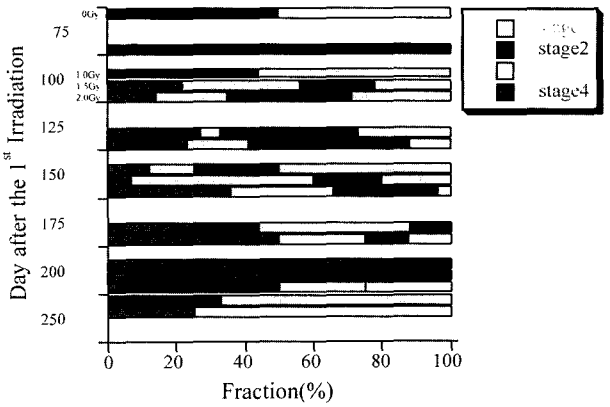

Fig. 10. Classification of histological stages of thymus.

pulmonary pathology preparation, I classified tumor of an organ of each or stage of stage of disease of a state of metastasis in 0-4. 0 shows that metastasis lesion is not recognized. We show a stage classification of a thymus of thymus lymphoma mice in figure 10 according to day by groups, dosage.

It is clear that a thymus lymphoma of a group is an early stage tumor in stage 1 or 2 in both dosages for 75 days. A ratio of stage three or four becomes high with increase of day instars in case of both dosages. Developing a thymus lymphoma each reduces it with six or seven mice in a group for group, 250 days for 200 days and cannot watch determination of a classification of a 4 stage. Determination increasing stage high of dosage is recognized in a group for group, 175 days for group, 150 days for group, 125 days for 100 days. We show a stage classification of spleen of thymus lymphoma mice in Figure 11 according to day by groups, dosage. Spleen

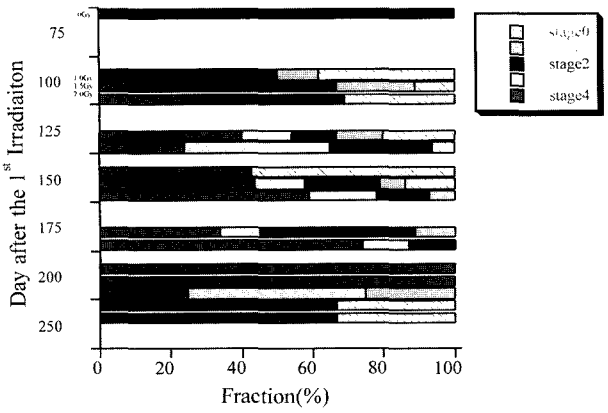

Fig. 11. Classification of histological stages of spleen.

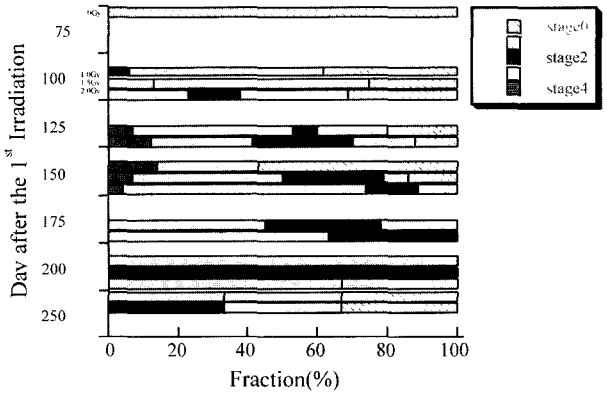

Fig. 12. Classification of histological stages of lung.

metastasis was recognized in a group on 75th by two of them of control group, and spleen metastasis is recognized in a group for 100 days by about 70% thymus lymphoma mice, and metastasis to spleen understands a remarkable thing after a group for 100 days. Because there is a little number of mice, a crowd cannot look at trend of a stage classification for 200, 250 days. That the high laboratory mouse which stage went along to of dosage increases in a group for group, 175 days for group, 150 days for group, 125 days for 100 days is recognized, and it is clear that there is dosage influence reference on stage of spleen. We show a pulmonary stage classification of thymus lymphoma mice in Figure 12 according to day by groups, dosage. Metastasis to lungs is not recognized in a group or 75th, and metastases to lung are recognized after a group for 100 days by 50% mice of and over. An organization of a thymus/spleen and the determination that the mice which stage went along to high one of dosage increases in the same way are recognized and understand that there is dosage influence reference on a stage

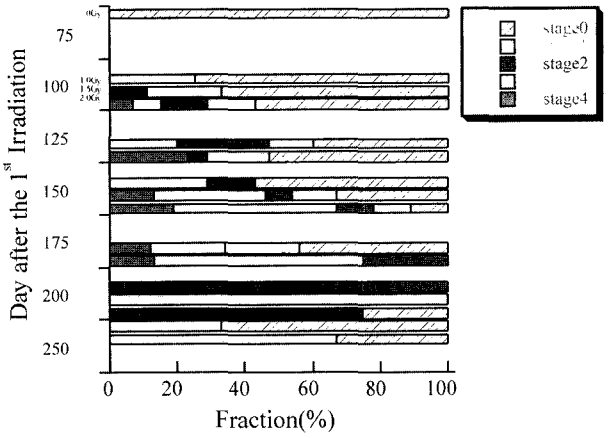

Fig. 13. Classification of higtological stages of liver.

classification of lungs. As for the thing in case of lungs with a little stage 4, as for stage 4 of a pulmonary organization, there become little tidal air areas, and it is natural that stage 4 is not recognized when it seems to be handling, this study, and sacrifice did a thing reaching death intentionally. We show an organization diagnosis result of liver of thymus lymphoma mice in Figure 13 according to day by groups, dosage.

A pulmonary organization and the determination that the high mice which stage went along to of dosage increases in the same way are recognized a thymus/spleen, and it is clear that there is dosage influence reference to every day group. In addition, in change of days every each dosage, following 1 with increase of day instars, and stage 3, a ratio of 4 become high. We resemble both dosage groups in a group for 100 days, and metastasis to liver is not recognized. It is clear metastasis to liver of a thymus lymphoma is late in comparison with spleen/lungs, and to occur.

A classification of degree of metastasis to lymph gland

Pathological by the degree of metastases to lymph nodes judge it macroscopically, and show the result that classed in stage 0 to 3 in Figure 14 according to day by groups, dosage. We show judgment basis of case of pathological diagnosis of lymph gland in appendix 2-4. Metastases to lymph nodes of a thymus lymphoma are not recognized in a group on 75th, and metastases to lymph nodes are recognized in a group for the first time for 100 days. There is much stage1 the mice that there is not metastasis has total haploid number in a group for group, 175 days for 150 days and when there is metastasis.

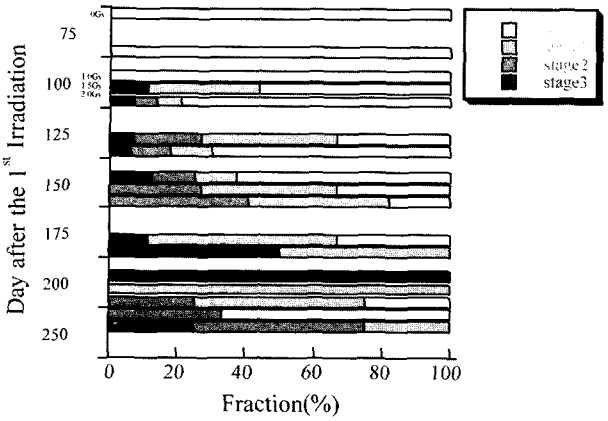

Fig. 14. Classification of pathological stages of the lymphnode.

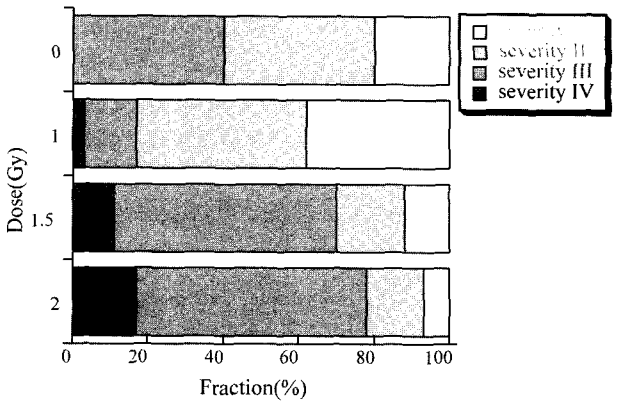

Fig. 15. Classification of severity of thymic lymphoma.

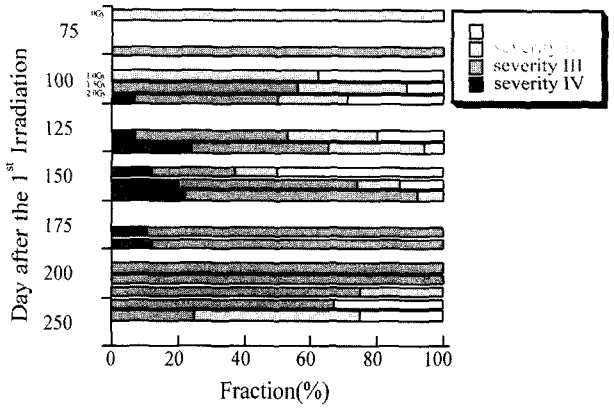

Fig. 16. Classification if severity of thymic lymphoma.

We compare it with a pulmonary organization diagnosis result, and determination stage of metastasis is late and progress of stage is late is recognized liver/spleen. Clear dosage influence relation is recognized in a 170 days group and 150 days group.

Pathologic anatomy findings and degree of seriousness and dosage reaction relation of a thymus lymphoma by histological observation

In degree of seriousness of a thymus lymphoma, we consider degree of invasion of tumor tissue in a thymus, proximal and remoteness lymph gland, degree of metastasis to liver, spleen and lungs and show the consequence which classified in Figure 15. We show detailed classification basis in appendix. Dosage reaction-related relation is recognized in degree of seriousness of a thymus lymphoma, and if it is it, it is accompanied, and high dosage understands that high degree of seriousness ratio of degree of seriousness increases. The mice that developed a thymus lymphoma of 0Gy is 5, and the reason why I occupy degree of seriousness 40% in 2 Gy is that there are a few and number of mice the internal degree of seriousness thing. In addition, we show the result that classed degree of seriousness in dosage distinction, day by groups in Figure 16. Dosage reaction reference of degree of seriousness is recognized in 175 days for group, 150 days group and 125 days group with much animal number of mice, and dosage is high, and a ratio of degree of seriousness mice increases.

Incidence and dosage reaction relation of a tumor except a thymus lymphoma

A main thing of a tumor except a thymus lymphoma is myeloid leukemia 13 examples (38.2%), lymphocytic leukemia 11 examples (32.4%), non-thymus nature lymphoma 8 examples (23.5%). Furthermore, it was admitted one example salivary gland neoplasm breast cancer in a group for one example, 1.5Gy, 175 days for 1.0Gy, 250 days by a crowd. And the laboratory mouse which died of a cause except a tumor is five of them pneumonia 2 (1.0Gy, 150 days, group) and cause of death lack of foresight are three of them. Hazard rate of a tumor except a thymus lymphoma with increase of dosage same as a thymus lymphoma, too and increased, and dosage dependence was recognized. I develop a tumor except a thymus lymphoma after a group for 100 days, and it is clear that the time of outbreak is late in comparison with a thymus lymphoma.

Discussion

In conclusion, the dose-effect relationship was observed regarding radiationinduced thymic lymphoma in C57BL/

6J mice. Our experimental results may be important in understanding the mechanism of radiation-induced cancer, and in considering the detriment in the radiological protection field. We attempt to ensure the reliability of these results by using immuno-staining of p53 protein in the next step of this experiment.

The time occurrence and the acceleration of radiation-induced Thymic lymphoma

Also, looking at the cancer in the time course showed that thymic lymphoma was first induced and seen in mice sacrificed at day 100 (130 days old), and then metastasize in the order of spleen, lung, liver and the lymphatic nodes.

The problems when using the life-long method in cancer research

Overmore, using the periodical sacrificing method as the observation method rather than the life-span observing method, we were able to obtain information concerning the outbreak of cancer, tumor growth, and the way of metastasizing.

The meaning of the dose-effect relationship in stochastic effects as in radiation protection

The serious radiation-induced effect of concern in radiation protection is divided into two categories: deterministic effects and stochastic effects. Since, deterministic effects has threshold doses and is easy to control, the point at issue in radiation protection field is the management of the stochastic effects. Stochastic effect, such as radiation-induced cancer, is defined as one in which the probability of the effect occurring increases continuously with increasing absorbed dose while the severity of the effect, in affected individuals, is independent of the magnitude of the absorbed dose (Muto *et al.*, 1983). And on this assumption many regulations dealing with radiological protection has been made. But, there has been some researches pointing out the fact that more severe skin cancer (squamous cell carcinoma) are observed in subjects who are exposed to higher doses. And, results of many animal experiments, read carefully from the dose-effect point of view, suggest the definition being not always true (Nuto *et al.*, 1987; Sado *et al.*, 1991).

The severity of the effect is one of the very important information, essential, in determining the detriment of radiation-induced effect holistically (Turusov 1979). Until now, the detriment of radiation-induced cancer has been calculated taking into account of the time.

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