Bull Yamaguchi Med School 54(1-2):1-7, 2007

# Thymic Architectures for T Cell Production and Structural and Functional Recovery from Their Radiation Injuries

*Tetsuo Fukumoto,*[1] *Yasuhiro Adachi,*[2] *Nobuko Tokuda,*[2] *Tomoo Sawada*[2] *and Yamini Arudchelvan*[3]

[1] Takehisa Hospital, Takehisa 2-53-8, Shimonoseki, Yamaguchi 751-0833, Japan
[2] Department of Organ Anatomy Yamaguchi University, Graduate School of Medicine, 1-1-1, Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
[3] Department of Basic Sciences Faculty of Dental Sciences University of Peradeniya, Peradeniya Sri Lanka
(Received March 12, 2007)

**Abstract** The thymus is an important organ for producing T cells. Thymic structures consist of thymic epithelial cells among which thymocytes proliferate and maturate. The structural and functional recovery of the rat thymus after irradiation was studied morphologically and by the message expression changes of several cytokines. In this manuscript we tried to describe further details of rat thymic epithelial cells in respect to their electron microscopic features as well as their function to interact with thymocytes. Then the mechanisms responsible for the regeneration of the thymus after irradiation were considered. Not only the reproliferation of the thymocytes but also the regeneration of the vasculatures and thymic epithelial cells are considered important for thymus regeneration. Cytokine message changes were shown to be seriously involved for the degree of recovery of the thymus tissue after irradiation.

*Key words:* thymus, thymic epithelial cells, irradiation, regeneration, cytokines

## 1. Introduction

The thymus is an important organ for producing T cells. The fine meshwork of the thymic epithelial cells (TECs) along the vascular structures consists of the hematopoietic microenvironments for thymocyte proliferation and maturation.[1][2] The detailed mechanisms of the thymocyte − thymic epithelial cell interactions for thymocyte proliferation and maturation in vivo are not elucidated yet, though progress has recently been made in elucidating events that regulate the development of intrathymic microenvironments.[2-4]

Phylogenically, typical thymus is observed from fish; the continuation of the epithelial cells of the endodermal origin makes the thymic epithelial cords among which thymocytes proliferate and maturate. The cortex consists of abundant thymocytes with cortical thymic epithelial cells, though the medulla consists of fewer relatively matured thymocytes with medullary thymic epithelial cells. Subsequently, the cortex appeared dark compared to the medulla in Hematoxylin and eosin staining. These typical structures are well conserved through the phylogeny. Whether thymic epithelial cell origin is either endodermal only or endodermal and ectodermal mixture, is still controversial.[5][6] Pax1 is a transcriptional regulatory protein and its expression in thymic epithelial cells can be detected through thymic development and in the adult.[7] Beside Pax1, Foxn1, a forkhead class transcription factor that is expressed by thymic epithelial cells is not required for the initial formation of the thymic primordium but it functions to regulate thymic epithelial

cell differentiation and proliferation.[8)9)] Furthermore, the keratinocyte growth factor (KGF) can significantly affect the development and function of thymic epithelial cells.[10)]

In this manuscript we tried to explain the morphological importance of the thymic epithelial cells to understand the interactions between thymocytes and thymic epithelial cells for thymocyte proliferation and maturation. We then considered the regeneration mechanisms for the thymus tissue after irradiation focusing on the thymic epithelial cell regeneration and message expression changes of several cytokines.

## 2. The morphology and function of the thymic epithelial cells

Light microscopically thymic epithelial cells can be discriminated from the thymocytes because of much content of the cytoplasm, and the relatively large and faintly stained nuclei. Following the electron microscopical features of the thymic epithelial cells, four types of the thymic epithelial cells were observed in the rat thymic cortex;[11)12)] subcapsular(perivascular), pale, intermediate and dark (Fig.1). Among these cells, subcapsular thymic epithelial cells are considered important for the proliferation of the immature type of thymocytes that originate from the bone marrow. On the other hand, pale thymic epithelial cells and probably intermediate thymic epithelial cells are thought to be the major thymic epithelial cells for thymocyte proliferation and maturation where positive selection may take place.[13)] The importance of immunoelectron microscopical analyses using antibodies against cell surface molecules and several enzymes for understanding the interactions between thymic epithelial cells and thymocytes were shown.[13–16)]

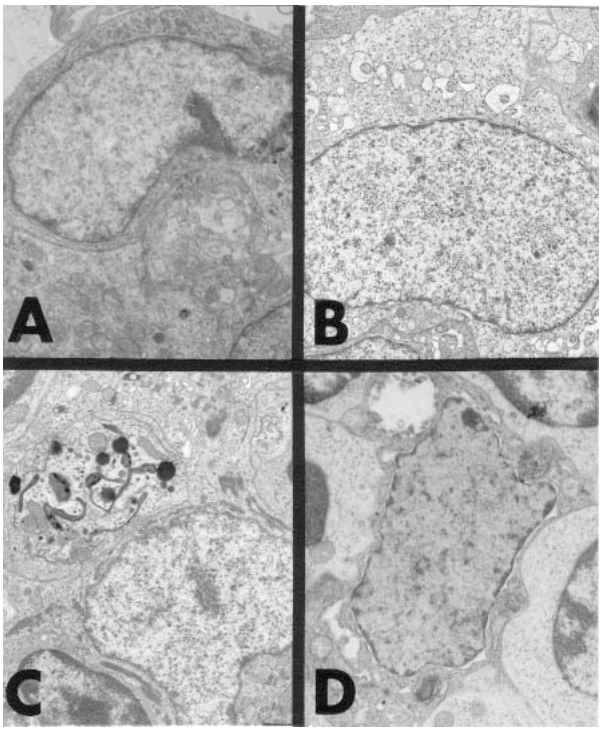


Fig. 1 Electron micrograph of rat thymic epithelial cell types.
A: a subcapsular (perivascular) thymic epithelial cell
B: a pale thymic epithelial cell
C: an intermediate-type cell
D: a dark cell
A x6,000 , B x7,500, C x6,000, D x6,700

## 3. Recovery from radiation injuries

Thymus is one of the radiosensitive organs. A great deal of research has been done[17)18)] but the detailed recovery mechanisms after irradiation have not yet been elucidated. We have studied the rat thymus after irradiation.[19)] The rats were irradiated with the radiation doses of 2 to 8 Gy. The changes in the body weights and the thymus weights were measured after irradiation.[19)20)] The thymus weights decreased and then recovered after irradiation. Figure 2 shows the changes of the thymus weights after irradiation. At doses of 2 to 6 Gy, these weights recovered quickly, though in the 8 Gy group, thymus recovered slowly and finally recovered only up to 65% of the normal thymus.[20)] Many thymocytes died and the percentages of thymocytes in the thymus decreased after irradiation. The double positive (CD4+CD8+) cell population, which is the major population of the thymocyte subsets (more than 80% in normal) decreased, but the double negative (CD4-CD8-) cell population which is the minor population of the thymocyte subsets (less than 1% in normal), increased.[21)22)] These percentage changes returned to almost normal on day 7 for 6 Gy irradiation and on day 14 for 8 Gy irradiation.

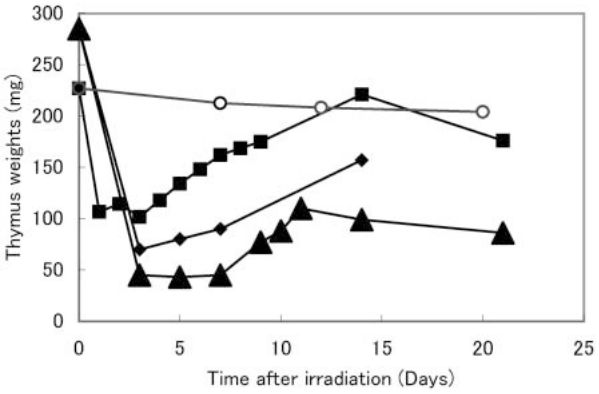


Fig. 2 Changes in thymus weights after several doses of irradiation.
Thymus weights (mg) were measured after irradiation. In the ranges of relatively low doses (2 Gy ▮—▮ and 6 Gy ◆—◆), thymus weights decreased once, then recovered rapidly, but in 8 Gy (▲—▲), thymus weights decreased, gradually increased, and eventually recovered only up to 65% of the normal thymus weights. Normal control (○—○).

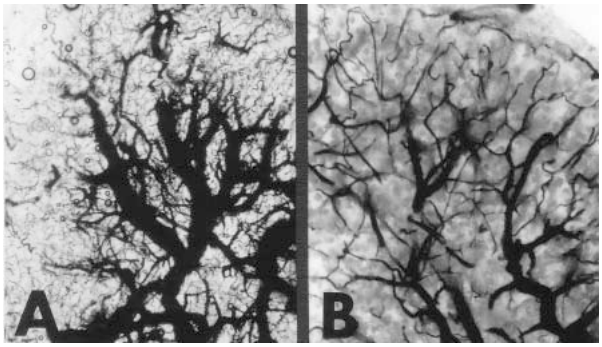


Fig. 3 Distribution of vasculature and its recovery after irradiation.
Rats were perfused with Indian ink and thymus tissue was observed under microscope. Vasculatures on day 3 after 6 Gy irradiation (A). The fine meshwork of the vessels in the cortex was destroyed, and extremely dilated vessels in the medulla were observed. On day 5 after irradiation (B), the fine meshwork in the cortex recovered, and dilatation of the medullary vessels returned to the normal sizes.
x 72

### 4. Vascular changes in the thymus after irradiation

The vascular structures were visualized with Indian ink. The unique fine meshwork in the cortex of the thymus was visualized in the thymus (Fig. 3). This fine vascular meshwork is indispensable, not only for thymic epithelial cells, but also for thymocytes. After irradiation, besides the severe damage to the thymocytes blood vessels showed severe changes following the irradiation doses. In the case of 6 Gy on day 3 after irradiation the fine vascular meshwork in the cortex disappeared and the extreme dilatation of the medullary vessels was observed[19] (Fig. 3A). These changes rapidly returned to almost normal on day 5 (Fig. 3B). The regeneration of the vascular endothelial cells and associated tissues may be accelerated with several cytokines released from the damaged thymic epithelial cells and maybe from the macrophages.

### 5. Radiation injury and repair

After irradiation, the thymocytes were destroyed. Many apoptotic figures of the thymocytes can be observed in the thymus in this stage (Fig. 4). Phagocytosed thymocytes by macrophages can be also observed.[23]

(A) Thymic epithelial stem cells may exist

In the 6 Gy irradiation on days 3 to 5 after irradiation, the percentages of macrophages increased, and they often phagocytosed dead thymocytes. On day 5 the percentages of thymocytes increased, and the structure of the thymus retuned to almost normal on day 7. Changes in the frequency of thymic epithelial cell subtypes were also observed.[12] Intermediate-type cells increased in frequency on days 3 to 5 after irradiation, and these changes returned to normal gradually. This type of epithelial cell may be an immature type. Furtheremore, in the 8 Gy irradiation, a frequency of immature types of p63 positive thymic epithelial cells increased. These results suggest that thymic epithelial cells may be reproduced after irradiation.[23] Subsequently, stem cells, or even not stem cells, some thymic epithelial cells having the potential to divide, may exist, particularly near the subcapsular region.

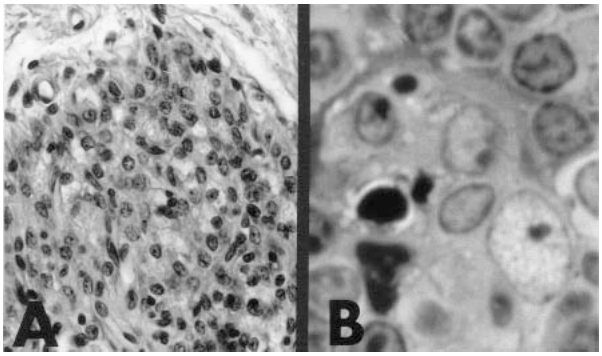


Fig. 4 The morphology of the irradiated and recovering rat thymuses.
Thymic epithelial cells are remarkably observed, even in the cortex in the thymus from the 10 Gy irradiated and recovering rat (day 7) (A).
Semithin sections stained with toluidine blue staining showed the reduced number of thymocytes in the irradiated and recovering rat thymus (6 Gy, day 7). Apoptotic figures of thymocytes are observed (B).
A x 390, B x 1,500

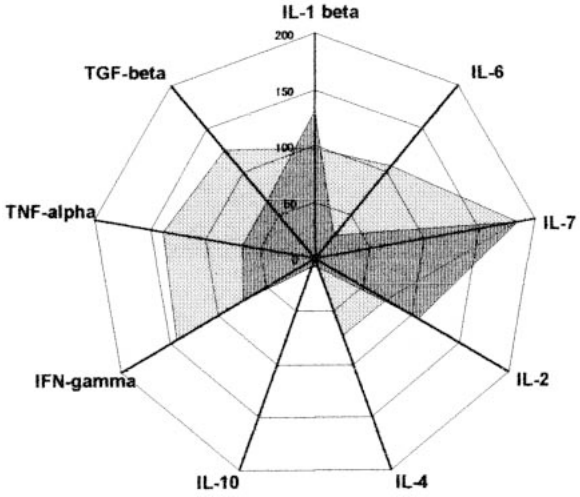


Fig. 5 The degrees of cytokine message expression were plotted for each 6 Gy and 8 Gy irradiation. Each cytokine gene expression was measured using RT-PCR on day 7 for 6Gy irradiation when thymic structures returned to almost normal, and on day 21 for 8 Gy irradiation when thymic structures returned to almost normal. The percent of recoveries compared to the normal levels were calculated and plotted on the graph. The gray area shows 6 Gy, and the dark gray represents 8 Gy irradiation.

(B) Cytokine changes may be important for the recovery

Cytokine message changes may be useful to understand the mechanisms responsible for the recovery of the thymus after irradiation. The message changes for the several cytokines were followed after irradiation.[24)25)] Some results were summarized in Fig. 5. As explained in Fig. 2, when the irradiation doses were as high as 8 Gy, the weights of thymus did not return to the normal level, but to only 65% of the normal weights, though, in 2 Gy and even 6 Gy the weights returned nearly to the normal level. The mechanisms concerning why the thymus weights did not return to normal level in 8 Gy irradiation compared to those in lower irradiation doses were not clear, but the possibility existed that thymic epithelial cell damages had been serious, and even after recovery thymic epithelial support for thymocyte proliferation and maturation may not be enough compared to the normal level. Cytokine message levels were compared between those for 6 Gy and 8 Gy irradiations (Fig. 5). These results were obtained after comparing for each cytokine levels when the histology of the thymus appeared almost normal on day 7 for 6 Gy, and on day 21 for 8 Gy after irradiation. In 8 Gy, TGF-$\beta$, TNF-$\alpha$, and INF-$\gamma$ appear ed less compared to 6 Gy, particularly IL-6, IL-10, and IL-4 were reduced. These results may reflect some structural alteration of thymus, particularly thymic epithelial cells after 8 Gy irradiation. Further analyses are required.

Summing up these results, the recovery process of the thymus after irradiation was considered (Fig. 6). Thymic epithelial cells and vascular structures consist of a fundamental structure for thymocyte proliferation and maturation. The details of the intimate interactions between thymic epithelial cells and thymocytes have been gradually elucidated in the molecular levels and by the immunoelectron microscopical analyses using antibodies against cell surface molecules.[13–16)] After irradiation, thymocytes were destroyed and phagocytozed by macrophages. Vascular

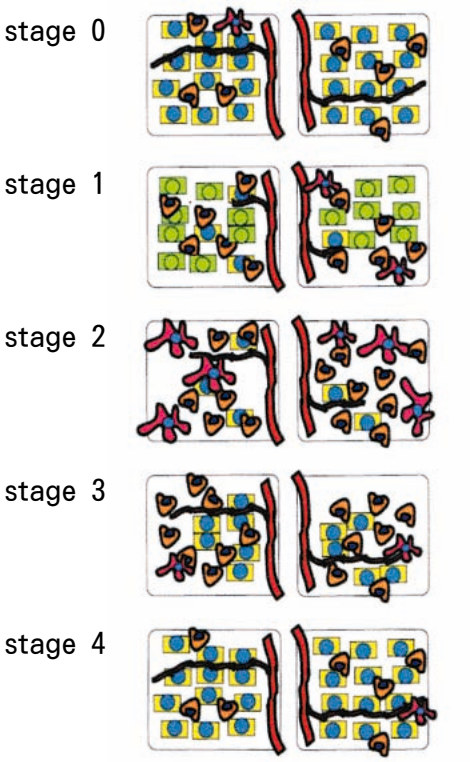


Fig. 6 Schematic representation of thymic changes after irradiation.

Stage 0: Thymocytes (yellow) filled the thymus among which thymic epithelial cells (orange) exist, providing the microenvironment for thymocyte proliferation and maturation. Vessels are well conserved.

Stage 1: After irradiation, most thymocytes died (green), and the fine meshwork of the blood vessels was destroyed. Thymic epithelial cells (orange) and macrophages (purple) remained.

Stage 2: Macrophages phagocytosed the dead thymocytes. Blood vessels started to recover. Thymocytes started to divide. Thymic epithelial cells may also start to proliferate if these have been damaged by enough doses of irradiation as 6 Gy and 8 Gy.

Stage 3: The preserved and recovered thymic epithelial cells and recovered blood vessels provide the suitable microenvironment to accelerate the proliferation and maturation of thymocytes. Relatively increased macrophages after irradiation reduced their number and keep the normal number in this stage.

Stage 4: A nearly recovered thymus.

structures also were destroyed. These changes evoke the cascade of several cytokine changes. Subsequently, a regeneration of vascular structures as well as the reproliferation and maturation of thymocytes take place. In the severely damaged thymus, as in the case of 8 Gy irradiation, damaged and destroyed thymic epithelial cells were replaced by the newly proliferated thymic epithelial cells, probably from thymic epithelial stem cells, which are thought to be p63 positive. Subsequently, after a reorganization of thymic microenvironment, the thymocytes will gain enough chances to proliferate and mature, although some structural and functional alterations after 8 Gy irradiation may affect the extent of thymocyte proliferation and maturation, eventually resulting in only 65% recovery in the thymic weights.

## Acknowledgements

The authors thank Messrs. M. Tamechika and A. Kumakura, for their valuable technical support. This study was supported by Grants-in-aid for Science Research from the Japan Society for the Promotion of Science (# 16591208).

## References

1) von Gaudecker, B.: The development of the human thymus microenvironment. *Curr. Topics Pathol.*, **75**: 1-41, 1986.

2) Fukumoto, T.: Precise analyses of the structure of the thymus for establishing details of the mechanisms underlying thymocyte proliferation and maturation. *Arch. Histol. Cytol.*, **60**: 1-8, 1997.

3) Anderson, G. and Jenkinson, E.J.: Lymphostromal interactions in thymic development and function. *Nat. Rev. Immunol.*, **1**: 31-40, 2001.

4) Gray, D.H.,Ueno,T.,Chidgey,A.P., Malin, M., Goldberg, G.L., Takahama, Y. and Boyd, R.L.: Controlling the thymic microenvironment. *Curr. Opin. Immunol.*, **17**: 137-143, 2005.

5) Haynes, B.F.: Human thymic epithelium and T cell development ; current issues and future directions. *Thymus*, **16**: 143-157, 1990.

6) Blackburn, C.C. and Manley, N.R.: Developing a new paradigm for thymus organogenesis. *Nat. Rev. Immunol.,* **4**: 278-289, 2004.
7) Wallin, J., Eibel, H., Neubuser, A., Wilting, J., Koseki, H. and Balling, R.: Pax1 is expressed during development of the thymus epithelium and is required for normal T-cell maturation. *Development,* **122**: 23-30, 1996.
8) Blackburn, C.C., Augustine, C.L., Li, R., Harvey, R.P., Malin, M.A., Boyd, R.L., Miller, J.F.A.P. and Morahan, G.: The nu gene acts cell-autonomously and is required for differentiation of thymic epithelial progenitors. *Proc. Natl. Acad. Sci. USA,* **93**: 5742-5746, 1996.
9) Su, D-M., Navarre, S., Oh, W-J., Condie, B.G. and Manley, N.R.: A domain of Foxn1 required for crosstalk-dependent thymic epithelial cell differentiation. *Nat. Immunol.,* **4**: 1128-1135, 2003.
10) Erickson, M., Morkowski, S., Lehar, S., Gillard, G., Beers, C., Dooley, J., Rubin, J.S., Rudensky, A. and Farr, A. G.: Regulation of thymic epithelium by keratinocyte growth factor. *Immunology,* **100**: 3269-3279, 2002.
11) de Waal, E.J. and Rademakers, L.H.P.M.: Heterogeneity of epithelial cells in the rat thymus. *Microsc. Res. Tech.,* **38**: 227-236, 1997.
12) Arudchelvan,Y., Tokuda, N., Tamechika, M., Wang, Y-H., Mizutani, N., Sawada, T., Yamaguchi, K., Fukumoto, T. and Shinozaki, F.: Semiquantitative morphological analysis of stromal cells in the irradiated and recovering rat thymus. *Arch. Histol. Cytol.,* **63**: 147-157, 2000.
13) Arudchelvan,Y., Nishimura,Y., Tokuda, N., Sawada, T., Shinozaki, F. and Fukumoto, T.: Differential expression of MHC classII antigens and cathepsin L by subtypes of cortical epithelial cells in the rat thymus; an immunoelectron microscopic study. *J. Electron Microsc.,* **51**: 173-181, 2002.
14) Arudchelvan,Y., Tokuda, N., Sawada, T., Shinozaki, F. and Fukumoto, T.: Spacial relation between major histocompatibility complex-restricted antigen receptor-bearing thymocytes and subtypes of thymic epithelial cells. *Anat. Rec.,* **267**: 131-136, 2002.
15) Arudchelvan, Y., Nishimura, Y., Tokuda, N., Ueyama, Y. and Fukumoto,T.: Identification and characterization of major histocompatibility complex class II compartments in cortical thymic epithelial cells. *Anat. Rec.,* **274**: 798-806, 2003.
16) Tokuda, N., Arudchelvan,Y., Sawada,T., Adachi, Y., Fukumoto, T., Yasuda, M., Sumida, H., Shioda, S., Fukuda, T., Arima, A. and Kubota, S.: PACAP receptor (PAC1-R) expression in rat and rhesus monkey thymus. *Ann. N.Y. Acad. Sci.,* **1070**: 581-585, 2006.
17) Takada, A., Takada,Y., Huang, C.C. and Ambrus, J.L.: Biphasic pattern of thymus regeneration after whole-body irradiation. *J. Exp. Med.,* **129**: 445-457, 1969.
18) Kadish, J.L. and Bash, R.S.: Thymic regeneration after lethal irradiation evidence for an intra-thymic radioresistant T cell precursor. *J. Immunol.,* **114**: 452-458, 1975.
19) Wang, H-Y., Tokuda, N., Tamechika, M., Hashimoto, N., Yamauchi, M., Kawamura, H., Irifune, T., Choi, M., Awaya, A., Sawada, T. and Fukumoto, T.: Stromal changes in irradiated and recovering rat thymus. *Histol. Histopathol.,* **14**: 791-796, 1999.
20) Tokuda, N., Katsube, K., Sakuragi, A., Nagato, S., Harada, D., Arudchelvan, Y., Mizutani, N., Wang, Y-H., Sawada, T., Fujikura, Y. and Fukumoto, T.: MHC class II antigen expression was different from MHC class I antigen expression in irradiated and recovering rat thymus. *Acta Histochem. Cytochem.,* **35**: 101-105, 2002.
21) Tsuchida, M., Konishi, M., Takai, K., Naito, K., Fujikura, Y. and Fukumoto, T.: Effects of irradiation, glucocorticoid and FK506 on cell-surface expression by rat thymocytes; a three colour flow cytofluorometric analysis. *Immunology,* **83**: 469-475, 1994.
22) Konishi, M., Takai, K., Jojima, K., Fujikura, Y., Naito, K. and Fukumoto, T.: Effects of FK506 on surface antigen expression by regenerating thymocytes after sublethal irradiation in the rat.

*Thymus,* **23** : 53-66, 1994.
23) Fujikura, Y., Wang, Y-H., Tsuchida, M., Konishi, M., Yamauchi, M., Kawamura, H., Sawada, T., Tokuda, N., Choi, M-K., Naito, K. and Fukumoto, T.: Morphoioigical and flow cytometrical analyses of regenerated rat thymus after irradiation. *Arch. Histol. Cytol.,* **60** : 79-87, 1997.
24) Irifune, T., Tamechika, M., Adachi, Y., Tokuda, N., Sawada, T. and Fukumoto, T.: Morphological and immunohistochemical changes to thymic epithelial cells in the irradiated and recovering rat thymus. *Arch. Histol. Cytol.,* **47** : 149-158, 2004.
25) Mizutani, N., Fujikura, Y., Wang, Y-H., Tamechika, M., Tokuda, N., Sawada, T. and Fukumoto, T.: Inflammatory and anti-inflammatory cytokines regulate the recovery from sublethal X irradiation in thymus. *Rad. Res.,* **157** : 281-289, 2002.
26) Adachi, Y., Tokuda, N., Sawada, T. and Fukumoto, T.: Semiquantitative detection of cytokine messages in X-irradiated and regenerating rat thymus. *Rad. Res.,* **163** : 400-407, 2005.