Differentiation and Maturation of Functional T Lymphocyte Subsets in the Thymus I. Effect of Irradiation of the Thymus

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SUMMARY

Effect of the irradiation of the thymus on the development of T cells was investigated. C57BL/6(H-2^b, Thy-1. 2) mice were irradiated without shielding or with shielding over the thymus or over the right leg and tail, and they were transferred with bone marrow cells from B10. Thy-1. 1 (H-2^b, Thy-1. 1) mice. After the reconstitution, the proportion of donor-type Thy-1. 1 bearing cells were analysed by flow cytofluorometry. In the whole-body irradiated mice thymus cells were nearly completely replaced by donor derived cells within 21 days. In thymus-shielded mice and right-leg-and-tail-shielded mice, about half of the thymus cells were donor-type cells at 28 days after the reconstitution.

The development of donor-derived cytotoxic T cell precursors (CTLp) and helper T cells (Th) in the thymus of these recipient mice was investigated. In order to increase the sensitivity of detection, concanavalin A-mediated polyclonal assay system was used for both CTLp and Th, and the PNA$^-$ cell fraction of thymocytes was used as a source of functional cells. In whole-body irradiated mice and in right-leg-and-tail-shielded and irradiated mice, CTLp developed before Th did, whereas the reverse was true in thymus-shielded and irradiated mice. These results strongly suggested that the capacity of the thymus to allow the proliferation of donor type Thy-1 bearing cells and the development of CTLp in the thymus was independent of whether or not this organ was irradiated. On the other hand, the development of functional Th was obviously retarded by the irradiation on the thymus, suggesting that the radiosensitive thymic element plays a critical role for the generation of Th.

INTRODUCTION

The thymus plays a major role in the differentiation and maturation of T lymphocytes. It has been shown that both cytotoxic (CTL) and helper (Th) T cells differentiate in the thymus (1), and the repertoire of these T cell subsets is influenced greatly by the phenotype of the major histocompatibility complex (MHC) of the thymus (2, 3). Recent observations, however, showed that the self specificity of MHC class I molecule-restricted CTL precursors (CTLp) was not significantly skewed toward the thymic haplotype (4-6), whereas the class II-restricted Th was profoundly skewed toward the I region phenotype of the thymus (7-10). Moreover, it was shown

that CTLp but not Th precursors can develop in the spleen of athymic nude mice or thymecto-mized, irradiated and bone marrow reconstituted mice (11, 12). These results indicated that not all T cell subpopulations were equally influenced by the MHC phenotype or microenvironment of the thymus.

In the previous study, we established a novel experimental system for quantitatively assaying the stem cells for T cell lineage in which part-body-shielded and irradiated mice were used as the recipients (14). In the same paper, we also showed that the proliferation of donor derived T cells in the thymus of recipient mice was not affected by the irradiation on the thymus. This finding suggested that the function of thymic milieu that supported the differentiation of stem cells to Thy-1 positive cells was radio-resistant. Steinman and Nussenzweig, however, showed that thymic dendritic cells which carried a high level of Ia antigen were quickly and markedly reduced in number following whole body irradiation (15). It was also shown that thymic antigen presenting cells which determined the self-Ia restriction of T cells were radio-sensitive and the turnover rate of these cells was dependent on the dose of radiation (16). Since the presence of Ia antigens in the thymus is shown to be prerequisite for the development of Th (17–19), the question remains whether or not the irradiation on the thymus affects intrathymic development of functional T cell subpopulations.

Taking advantages of part-body irradiation (14) and by using highly sensitive and efficient assay systems for CTLp and Th, we investigated the effect of irradiation on early intrathymic development of CTLp and Th from donor bone marrow cells. The results showed that the functional development of Th in the thymus was markedly retarded by the irradiation on the thymus, whereas the generation of CTLp was less affected by the irradiation.

MATERIALS AND METHODS

Mice

The B10. Thy-1. 1 (H-2^b, Thy-1. 1) strain was established and kindly donated by Dr. T. Sado (National Institute of Radiological Sciences, Chiba, Japan). C57BL/6 (B6, H-2^b, Thy-1. 2) mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). These strains were bred and maintained in our animal facilities, and mice were used at 8 to 12 weeks of age.

Irradiation of mice

Whole body irradiation of mice were performed with a therapeutic ^{60}Co γ-ray source (Theratron 780 ; Atomic Energy of Canada Ltd., Ottawa, Canada) at the rate of about 55R/min. In thymus-shielding experiments, irradiation was performed with a therapeutic X-ray emmiter (Toshiba Electric Co., Tokyo, Japan) at 200 kV and 25 mA with an added filter of 0. 5 mm copper and 0. 5 mm aluminum at the rate of 55 R/min. Part-body shielding was performed as follows. For shielding from γ-rays, mice were anesthetized and settled on a plastic board with vinyl tape. The portion of the body to be shielded was covered with plastic board, and a lead block of 12. 5 cm thick was set on the plastic board. In thymus shielding experiments, anesthetize mice were settled on the bottom of a plastic box with the depth of 1. 5 cm. A lead bar of 1. 5 cm wide and 1. 0 cm thick was laid over the upper portion of the chest covering the thymus to

protect it from X-ray.

Monoclonal antibodies and flow cytofluorometry

Hybridoma cell lines, T-11-D7(anti-Thy-1. 1) and HO-13-4(anti-Thy-1. 2) were donated by Dr. H. Ishikawa (Keio University, Tokyo, Japan). The purification of antibody from ascitic fluids, conjugation of fluorescein isothiocyanate (FITC) to these antibodies and flow cytometric analysis of thymocytes by Ortho Spectrum III (Ortho Diagnostic systems, Westwood, MA) were described precisely in the previous paper (14).

Cell suspensions and transfer

Suspensions of spleen, thymus or bone marrow cells were prepared as described in the previous paper (14). Bone marrow cells were routinely treated with anti-Thy-1. 1 antibody (1/1000 dilution of T-11-D7 ascitic fluid) and complement (C, 1/10 dilution of selected rabbit serum). Cells were transferred to recipient mice by i. v. injection via tail vein within 3 hr after irradiation, and transfer was carried out only in sex-matched combinations.

Reagents

Con A was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and phytohema-gglutinin-M (PHA-M) and lipopolysaccharide (LPS 055:B5 from *Esherichia coli*) were from Difco Laboratories, Inc. (Detroit, MI). Peanut agglutinin (PNA) isolated by the method of Lotan et al. (20) was kindy donated by Dr. S. Terada (Fukuoka University, Fukuoka, Japan). α-methyl-D-mannoside (α-MM) and D-galactose were obtained from Wako Pure Chemical Co. (Osaka, Japan).

Fractionation of thymus cells with peanut agglutinin

PNA high-binding (PNA$^+$) and low-binding (PNA$^-$) fractions of thymus cells were obtained by the method originally described by Reisner et al. (21). Briefly, $2-3\times10^8$ thymus cells in 0. 2 ml of Eagle's minimum essential medium (MEM, Nissui Seiyaku Co., Tokyo, Japan) were mixed with an equal volume of PNA (1 mg/ml in PBS), and the mixture was incubated for 10 min at room temperature. Cells were then loaded on the top of 10 ml of MEM containing 25% heat inactivated fetal calf serum (FCS, M. A. Bioproducts, Walkersville, MA.) and left stand for 20 min at room temperature. Nonagglutinated cells in the top 2 ml of MEM and agglutinated cells in the bottom were collected separately and wahed two times with 0. 2 M D-galactose in MEM to remove the cell-bound PNA.

T cell growth factor (TCGF)

As a source of T cell growth factor, the supernatant of Con A-stimulated rat spleen cell culture was obtained and fractionated by Sephadex G-100 as described by Gillis et al. (22). Fractions having the costimulator activity, the ability to augment mitogen-induced thymocyte mitogenesis (23), were collected and used as partially purified TCGF.

Limiting dilution assay for CTLp

Frequency of polyclonal CTLp was assayed by the method of Wilson et al. (24) with slight modifications. The medium used for the culture was Iscove's modified Dullbecco's medium (Grand Island Biological Co., Grand Island, NY) containing 10% FCS, 2-mercaptoethanol (5×10^{-5} M), penicillin (100 U/ml) and streptomycin (100 μg/ml). Varying numbers of responder thymus cells were cultured in the flat-bottomed microtest plate (Coster, 3596) together with 5×10^5 X-

irradiated (3500R) B6 spleen cells as filler cells in 0. 2 ml volume of medium containing 3 μg/ml of Con A and 5% of rat TCGF. Cells were incubated at 37°C for 7 days in a CO_2 incubator (5% CO_2 in air). Twenty four microcultures were set up for each dose of responder cells. Cultures of thymus cells from normal B10. Thy-1. 1 mice were also set up as the control responder cells. After culture, lection (PHA)-mediated polyclonal cytotoxic activity was measured by the ^{51}Cr release test using P. 15 mastocytoma cells as targets (25). Fifty μl of medium containing 10^4 of ^{51}Cr-labelled P815 cells and 4%PHA-M was added to each well and the plates were incubated at 37°C in a CO_2 incubator. Five hours later, 0. 1 ml of supernatant was harvested and ^{51}Cr release was measured by γ-countor. Culture wells with higher ^{51}Cr release than the mean spontaneous ^{51}Cr replease plus 3 standard deviation regarded as positive for CTLp acitvity. In all experiments, target cell lysis was not observed in the control cultures which contained only X-irradiated B6 filler cells. The statistical analysis of CTLp frequency was calculated by the formula in Wison et al. (24).

Assay for Con A-mediated helper activity

Con A-mediated helper activity was assayed as polyclonal induction of B cells to immuno-globulin (Ig) production as previously described (26, 17). Breifly, varying numbers of thymus cells or splenic T cells were mixed with 5×10^4 splenic B cells (anti-Thy-1. 2 plus C treated spleen cells). To this mixtures, Con A was added at a final concentration of 5 μg/ml. As the controls, cell mixtures without Con A, B cell suspensnions with Con A, and B cell suspensions with LPS (50 μg/ml) were prepared. Cells were cultured triplicate in microtest plate (Coster, 3596) in 0. 2 ml volume of RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, penicillin (100 U/ml) and streptomycin (100 μg/ml). Five days after culture, cells were harvested and IgM secreting cells were enumerated by the protein A plaque assay as previously descrived (28).

RESULTS

Development of donor bone marrow-derived T cells in the thymus of part body-shielded and irradiated mice or whole body irradiated mice

B6 mice were shielded over the thymus and irradiated with 900R X-ray, or they were shielded over the right leg and tail and irradiated with 900R γ-ray. Mice were also whole-body irradiated with 800R γ-ray. These mice were transferred with 1. 5×10^7 bone marrow cells from B10. Thy-1. 1 mice. The proportion of donor-type Thy-1. 1 positive cells in the recipient thymus cells was measured by flow cytofluorometer on day 28 after the reconstitution, and the results are shown in Figure 1. In thymus-shielded and irradiated mice or right-leg-and-tail-shielded and irradiated mice, about half of the recipient thymocytes were replaced by donor derived cells. In whole-body irradiated mice, the recipient thymocytes were almost completely the donor type cells. As pointed out in the previous paper, part-body shielded and irradiated mice were effective in quantitatively analysing the stem cells for T cell lineage, because in these mice T cells were not completely replaced by donor derived cells. It was also shown that the irradiation of the recipient's thymus is not necessary condition for donor stem cells to enter the thymus and to differentiate to Thy-1 positive cells.
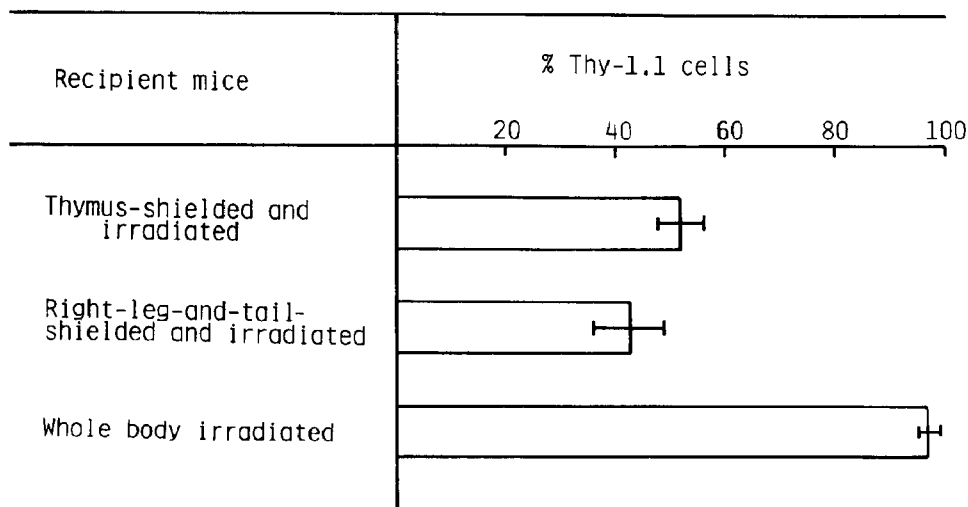
Recipient mice	% Thy-1.1 cells
	20 40 60 80 100
Thymus-shielded and irradiated	
Right-leg-and-tail-shielded and irradiated	
Whole body irradiated	

Fig. 1. Development of donor bone marrow-derived T cells in the thymus of part-body-shielded and irradiated mice or whole-body irradiated mice. B6 mice were shielded over the thymus and irradiated with 900R X-ray, or shielded over the right leg and tail and irradiated with 900R γ-ray, or whole-body irradiated with 800R γ-ray. These mice were injected i. v. with 1.5×10^7 bone marrow cells from B10. Thy-1.1 mice, and the proportion of Thy-1.1$^+$ cells in the recipient's thymus was measured on day 28 after reconstitution. Results are the mean of four mice$\pm$S. E.

Con A-induced polyclonal cytotoxicity and polyclonal helper T cell activitiy of thymus cells

For the analysis of functional development of T cells in the thymus, highly sensitive and efficient assay system is required. In this respect lectin-mediated polyclonal assay system has been used as powerful tools in the analysis of function of normal unprimed T cells (26, 27, 24). Such polyconal assay systems are expected to overcome the restriction of clonal specificities of lymphocytes, and the magnitude of response is higher than that obtained by antigen specific expression of the function. Thus we tried to apply the concanavalin A-mediated assay system in this study in order to assess the development of CTLp and Th activities in the thymus. Thymus cells from B6 mice were either unfractionated or fractionated into PNA$^-$ and PNA$^+$ populations. The frequency of polyclonal CTLp was determined by the limiting dilution assay in which varying numbers of thymus cells were cocultured with irradiated filler cells in the presence of optimal dose of Con A and rat TCGF. CTL activity of each culture was assessed by PHA-mediated polyclonal cytotoxic assay as described in Materials and Methods. For assessing Th activity of thymus cells, varying numbers of unfractionated or PNA-fractionated thymus cells were cocultured with normal splenic B cells in the presence of Con A, and the number of IgM-secreting cells (PFC) was enumerated by the protein A plaque assay. Results are shown in Figure 2.

Both unfractionated and PNA$^-$ fraction of thymus cells were shown to contain CTLp, and the frequency of CTLp in PNA$^-$ cell fraction was much higher than that in unfractionated thymocytes. CTLp in PNA$^+$ fraction may be due to a small number of PNA$^-$ cells contaminated in PNA$^+$ fraction. Th activity was observed also in both unfractionated and PNA$^-$ thymus cells, and the activity in PNA$^-$ cells is several-fold higher than that of unseparated thymus cells. No Ig production was seen when the PNA$^+$ thymus cells were used as helper source. These results indicated that both assay methods used in the present work for CTLp and Th were

Number of thymus cells (×10⁻³)
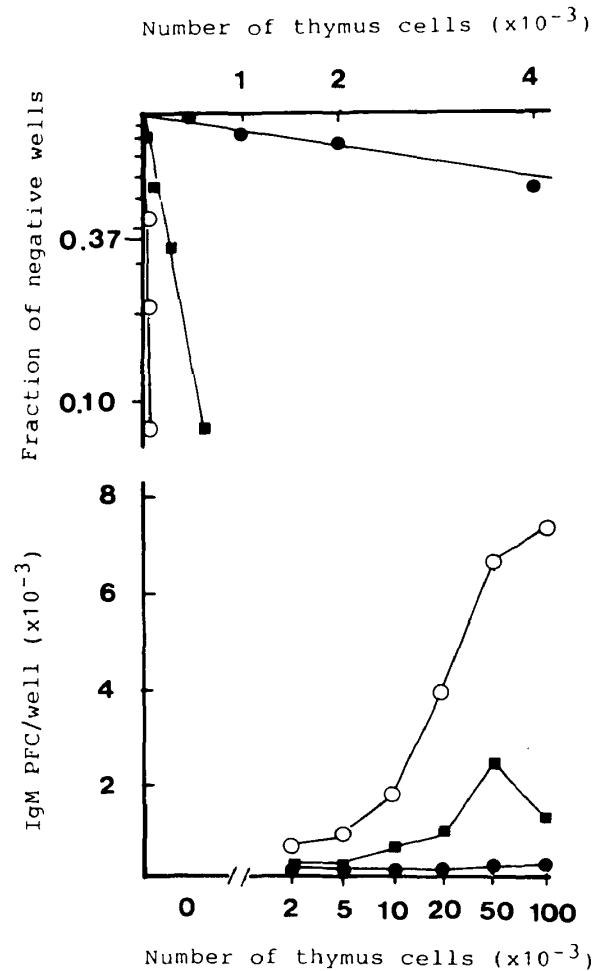

Number of thymus cells (×10⁻³)

Fig. 2. Con A-induced polyclonal cytotoxic and helper T cell activities of thymus cells. Thymus cells from B6 mice were either unfractionated (■) or fractionated into PNA⁻ (O) and PNA⁺(●) fractions. Upper panel : The frequency of polyclonal CTLp as determined by the limiting dilution assay in which varing numbers of thymus cells were cocultured with 3500R X-ray irradiated filler cells (5×10⁵ of B6 spleen cells) in the presence of Con A (3 μg/ml) and rat TCGF (5%). Twenty four microcultures were set up for each cell dilution and 7 days later CTL activity of each culture was assessed by PHA-mediated cytotoxic assay as described in MATERIALS AND METHODS. Lower panel : Varing numbers of unfracitonated or fractionated thymus cells were cocultured with normal splenic B cells (5×10⁴) in the presence of Con A (5 μg/ml), and 5 days later the number of IgM-secreting cells was enumerated by the protein A plaque assay.

effective in detecting the activity of a small number of functional thymus cells, and that almost all the activities of thymus cells were carried by PNA⁻ fraction. In the following experiment, only PNA⁻ fraction was investigated for CTLp and Th.

Development of PNA⁺ and PNA⁻ donor type cells in whole-body irradiated or thymus-shielded and irradiated mice

The time course of the development of functional (PNA⁻) or non-functional (PNA⁺) thymus cells in whole-body irradiated mice and in thymus-shielded and irradiated mice was investigated. Various intervals after irradiation and reconstitution with 1.5×10⁷ bone marrow cells, thymus cells were harvested and fractionated into PNA⁻ and PNA⁺ populations. The proportions of donor type Thy-1.1⁺ cells in each fraction were determined and shown in Figure 3. In whole-
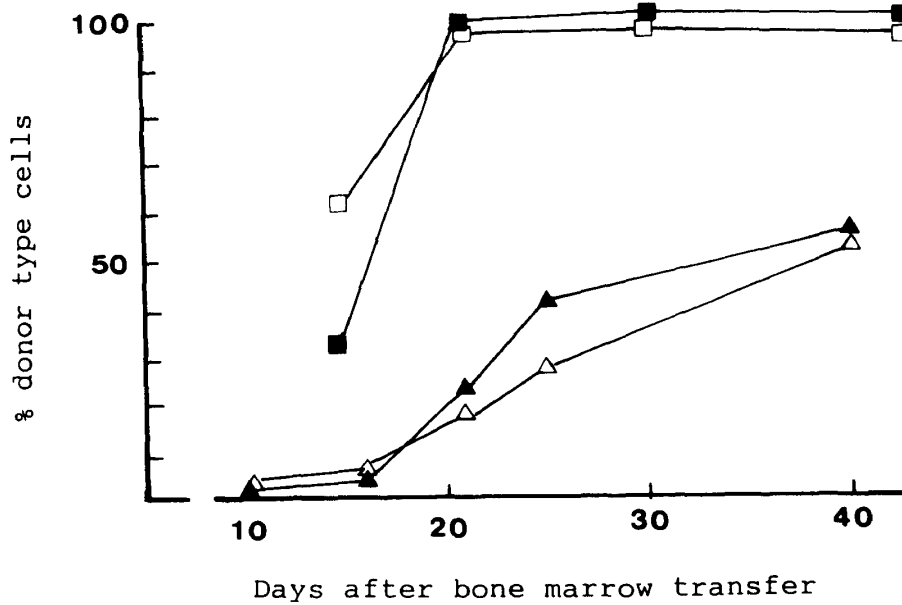
Days after bone marrow transfer

Fig. 3. Development of donor type Thy-1. 1$^+$ cells in PNA$^+$ and PNA$^-$ fractions in whole-body irradiated or thymus shielded and irradiated mice. B6 mice were either whole-body irradiated with 800R γ-ray, or thymus-shielded and irradiated with 900R X-ray and 1.5×10^7 bone marrow cells from B10. Thy-1. 1 donor mice were transferred. Various days after reconstitution, the recipient thymus cells were harvested and fractionated into PNA$^+$ and PNA$^-$ populations. The proportions of donor type Thy-1. 1$^+$ cells in PNA$^+$ (■) or PNA$^-$ (□) cells of whole-body irradiated mice and in PNA$^+$ (▲) or PNA$^-$ (△) cells of thymus-shielded and irradiated mice were assessed by flow cytofluorometry.

body irradiated mice, both PNA$^-$ and PNA$^+$ cells of donor origin began to repopulate the recipient's thymus about 2 weeks after reconstitution, and the thymus was completely replaced by donor derived cells by the third week. In thymus-shielded and irradiated mice, donor derived T cells were first observed 2 weeks after reconstitution and the level gradually increased. The maximum value of repopulation was achieved at about sixth week, when nearly half of the each fraction was replaced by donor type cells.

Development of CTLp and Th activities in whole-body irradiated mice

Whole-body irradiated B6 mice were injected with 1. 5×10^7 B10.Thy-1. 1 bone marrow cells. Various days after reconstitution, donor-type PNA$^-$ thymus cells were purified and treated with anti-Thy-1. 2 plus C to remove recipient-type cells. The resulting cells were assayed for CTLp and Th activities. PNA$^-$ thymus cells from normal B10.Thy-1. 1 thymocytes were served as a positive control. As shown in Table 1, both CTLp and Th activities were detectable as early as day 15, when donor derived cells were quickly increasing (Figure 3). CTLp frequency increased consistently up to day 25 when the frequency was more than two times higher than control thymus cells, and thereafter CTLp activity gradually decreased to normal control levels. On the other hand, Th activity was significantly lower than control levels (19. 6-37. 6% of control levels) up to day 30, and slowly increased thereafter. These results may indicate that CTLp develops faster than the precursor of Th in whole-body irradiated recipient mice.

Development of CTLp and Th activities in thymus-shielded and irradiated mice

The reason why the development of Th activity was delayed in whole-body irradiated mice

Table 1. Development of CTLp and Th in PNA⁻ fraction of thymus cells in 800R whole body irradiated mice.

| Days after transfer | CTL activity[a] | | Th activity[b] | |
	CTLp frequency (control)[c]	$\frac{\%}{\text{control}}$	PFC/well (control)[c]	$\frac{\%}{\text{control}}$
15	0.03 (0.11)	27.9	866±185(2800±115)	30.9
21	0.06 (0.20)	31.5	466±185(2400±288)	19.4
25	0.33 (0.14)	232.8	n.d.[d]	
30	0.13 (0.17)	74.9	1566±240(4166±204)	37.6
42	n.d.		2700±346(4633±233)	58.2
60	0.06 (0.08)	75.0	n.d.	

[a] Frequencies of CTLp were determined by the limiting dilution assay as described in MATERIALS AND MFTHODS.

[b] PNA⁻ thymocytes (10^5) were cultured with 5×10^4 splenic B cells in the presence of Con A, and IgM-secreting PFCs were enumerated 5 days later.

[c] Response of PNA⁻ thymus cells from normal mice.

[d] not done.

Table 2. Development of CTLp and Th of donor origin in PNA⁻ fraction of thymus cells from thymus-shielded and 900R irradiated mice.

| Days after transfer | CTL activity[a] | | Th activity[b] | |
	CTLp frequency (control)[c]	$\frac{\%}{\text{control}}$	PFC/well (control)[c]	$\frac{\%}{\text{control}}$
10	n.d.[d]		650±150(2566± 33)	25.3
15	0.03 (0.11)	31.5	1166±120(2800±115)	41.6
21	0.03 (0.10)	14.0	2530±145(3433±240)	73.5
25	0.03 (0.14)	21.8	3333±176(3330±280)	100.0
60	0.10 (0.08)	125.0	n.d.	

[a] Frequencies of CTLp were determined by the limiting dilution assay as described in MATERIALS AND METHODS.

[b] PNA⁻ thymocytes (10^5) were cultured with 5×10^4 splenic B cells in the presence of Con A, and IgM PFCs were enumerated 5 days later.

[c] Response of PNA⁻ thymus cells from normal mice.

[d] not done

is not clear. It was probable that irradiation of thymus resulted in the functional defect of thymic microenvironment which supports the development of Th but not of CTLp. To test this possibility, thymus shielding experiment was performed. B6 mice were thymus-shielded and irradiated (900R) and reconstituted with B10.Thy-1. 1 bone marrow cells. Various days after reconstitution, donor type PNA⁻ thymus cells were obtained and assayed for CTLp and Th activities. As shown in Table 2, the development of CTLp in thymus-shielded and irradiated mice was very low up to 25 days after reconstitution and developed to the normal control levels at around 60 days. In contrast, Th activity increased consistently during first 3 weeks and reached the normal control levels at about day 25. These results indicate that under the unirradiated thymic microenvironment, Th precursors develop faster than CTLp. This order of development is the same as that of normal ontogenetic development of Th and CTLp (29-31).

Comparison of the development of CTLp and Th in thymus-shielded or right-leg-and-tail-shielded and irradiated mice

Table 3. Comparison of CTLp and Th activities in PNA⁻ fraction of thymus
cells of donor origin developed in thymus-shielded or right-leg-and-
tail-shielded 900R irradiated mice.[a]

Shielded portion	CTL activity[b]		Th activity[c]	
	CTLp frequency	$\frac{\%}{control}$	PFC/well	$\frac{\%}{control}$
Non irradiated[d]	0.17	100.0	2660±145	100.0
Right leg and tail	0.15	88.2	766±167	28.7
Thymus	0.04	23.5	2100±173	80.7

[a] 28 days after the irradiation and reconstitution.

[b] Frequencies of CTLp were determined by the limiting dilution assay as described in MATERIALS AND METHODS.

[c] PNA⁻ thymus cells (1×10^5) were cultured with 5×10^4 splenic B cells in the presence of Con A, and IgM PFCs were enumerated on day 5.

[d] Non-irradiated normal B6 mice.

In the previous sections, the development of CTLp and Th precursors was comparatively studied in whole-body γ-ray irradiated mice and thymus-shielded and X-ray irradiated mice, and we reached the idea that nonirradiated thymus was more preferable for the development of Th precursor than irradiated thymus. A possibility remains, however, that the difference in the developmental features of CTLp and Th may be ascribed to the difference in the source of radiation. In order to check this possibility, the recipient B6 mice were shielded over the right leg and tail or over the thymus, and these mice were irradiated with 900R X-ray, then reconstituted with bone marrow cells from B10. Thy-1.1 mice. Both CTLp and Th activities were assayed 28 days later, and the results are shown in Table 3. In right-leg-and-tail-shielded and irradiated mice, CTLp activity was nearly the levels of normal control mice (88.2%), whereas Th activity remained considerably low (28.7%). On the other hand, in thymus-shielded and irradiated mice CTLp activity was low (23.5%), whereas Th activity was comparable to the control levels (80.7%). These results, together with those in the previous sections, indicate that the developmental pattern of functional CTLp and Th precursors in right-leg-and-tail-shielded and irradiated mice is similar to that in whole-body irradiated mice. From these observations, it was strongly suggested that the raddiation senstive thymic microenvironment is indispensable for the development of functional Th cell subset but not CTLp subset. It was further suggested that intact thymic microenvironment could be suppressive on the early development of functional CTL precursors.

DISCUSSION

The present study showed that the irradiation on the thymus did not affect the rate of generation of donor type T cells in the recipient's thymus (Figure 1), whereas the same treatment was shown to reduce the differentiation of Th cells (Table 1 and 3). In the thymus-shielded and irradiated mice the activity of Th cells matured to the levels of normal mice by 25 days after the irradiation and marrow reconstitution (Table 2). However, in cases in which the the thymus was exposed to irradiation, whole-bdoy irradiated recipients (Table 1) and leg-and-tail-shielded recipients (Table 3), development of functional Th activity was markedly reduced. In contrast,

CTLp developed much earlier in whole-body irradiated recipients than in thymus-shielded recipients. Since it was shown in normal ontogeny that Th developed earlier than CTL (29-31), the delay in the development of Th in the mouse whose thymus was irradiated may indicate that thymic microenvironment which is necessary for the functional development of Th but not for CTLp was injured by irradiation.

Development of functional Th is shown to decrease in mice continuously treated with anti-Ia antibody beginning from the new born age(32). Such treated mice exhibited reduced Ia antigen expression in the thymus (33). Steinman and Nussenzweig have shown that thymic dendritic cells, which express a high level of Ia antigen, were quickly and markedly reduced in number after whole body irradiation (15). In addition, it was shown that thymic antigen presenting cells which determine the self-Ia restriction of Th were radio-sensitive and bone marrow origin, and the turnover of these cells in the radiation-induced bone marrow chimera was dependent upon the dose of radiation (16, 17). We have recently observed that the density of Ia antigen expression in the medullary area of the thymus was markedly reduced by whole-body irradiation but not by irradiation with thymus-shielding (to be published).

Development of CTLp does not so strictly depend on the thymic microenvironment as the case of Th precursors. For example, CTLp are shown to develop in the spleen of athymic nude mice (11, 12) or in the thymectomized, irradiated and bone marrow reconstituted mice (13). These results do not, however, deny any participation of the thymus in the functional maturation of CTL. For example, Zinkernargel's original work clearly showed the importance of thymus epithelial cells in the MHC-restricted antigen recongnition by CTL (32). Furthermore, it has been shown that class I molecules of MHC in the thymus exert the great influences on the specificity repertoire of CTLp (4-6) or some differences in the CTLp prepertoire are observed between athymic nude mice and normal euthymic mice (12). Present study showed that the development of CTLp in the thymus was much accelerated by the irradiation on the thymus (Table 1 and 3). This is in contrast to the delay in the development of Th presursors in the same irradiated thymus. As discussed in the preceding section, the development of Th cells may depend upon the Ia positive cells in the thymus. It is unclear, however, whether the reverse is true for CTLp, namely that the presence of Ia positive cells in the thymus suppresses the development of CTLp. Studies are in progress to clarify whether the irradiation on the thymus affects the proportion of L3T4$^+$ cells versus Lyt-2$^+$ cells, or it affects only the functional maturation of these cells.

In order to further investigate the precise mechanism of T cell differentiation, it is necessary to establish an experimental system in which a single precursor for T cell lineage can differentiate into functional T cell subsets. This project is in progress by using intrathymic injection of limited number of bone marrow cells to irradiated mice. With this system, we currently investigating whether a single stem cell for T cell lineage can differentiate to functional T cell subsets, or whether and how the thymic microenvironment affect the sebsequent development of CTLp and Th functions. A study of similar aim is also in progress by using in vitro organ culture system (33).

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