Antileukemic Effect of Interleukin-2 on Spontaneous Development of Leukemia after H-2-Compatible Allogeneic Bone Marrow Transplantation in AKR/J Mice

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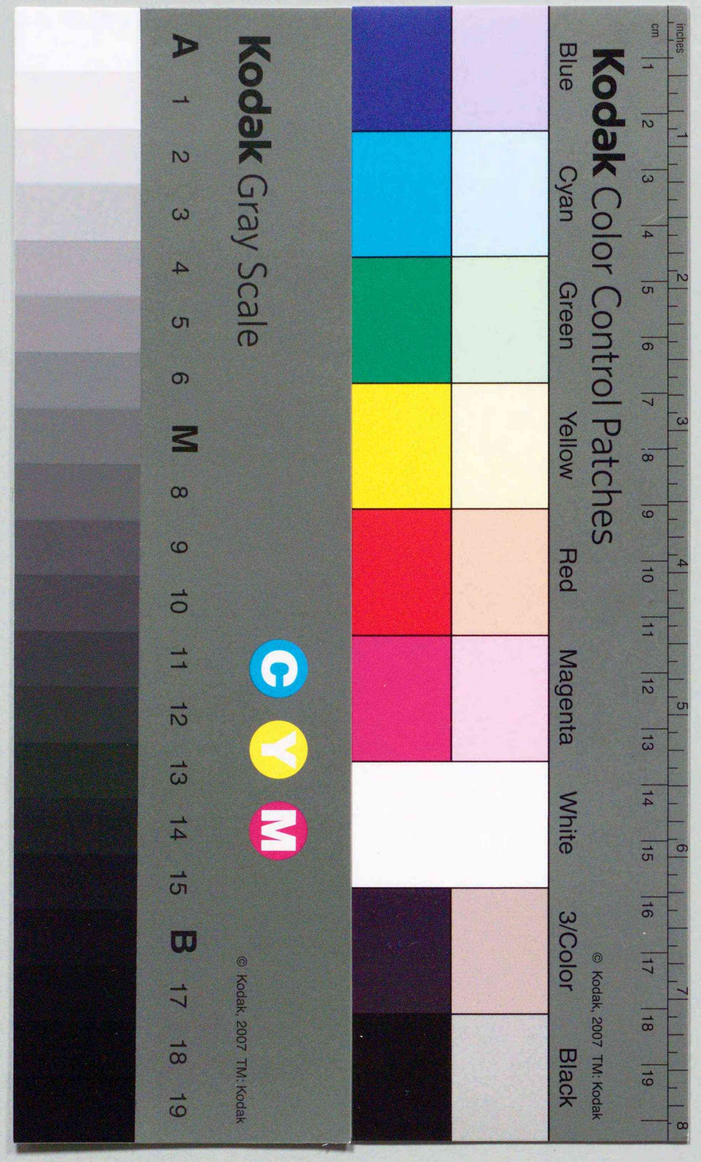

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Running Title : Prevention of leukemia in AKR by IL-2

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Summary

AKR/J mice, highly susceptible to spontaneous T cell leukemogenesis, were protected from developing the disease by H-2-compatible allogeneic bone marrow transplantation (BMT) and intermittent treatment with interleukin-2 (IL-2). Allogeneic BMT from C3H/HeJ mice and treatment with PBS yielded T cell leukemia in chimeras after the same latent period as that observed in normal AKR/J mice. In contrast, IL-2-treated chimeras caused an incidence of only 40% T cell leukemia. The preventing effect of IL-2 on leukemia development was not observed in one-year-treated chimeras, probably due to a lack of continuous antileukemic effects over the long term. Both LAK and NK activities in spleen cells were significantly increased in IL-2-treated chimeras. The cytotoxicity against T cell lymphoma cell line derived from AKR/J also increased in the IL-2-treated chimeras. Similarly, LPS-, PWM-, and IL-2-induced responses were increased in the IL-2-treated chimeras. TNF-alpha secretion from spleen cells also rose after IL-2 administration. IL-1 beta, IFN-gamma, and TNF-alpha mRNA became detectable in spleen cells using the PCR technique. The characteristics of leukemia cells in chimeras with overt leukemia were not directly affected by IL-2 administration. It is suggested that partial inhibition of spontaneous T cell leukemia development in AKR/J mice by allogeneic BMT and IL-2 may be due to the enhancement of graft-versus-leukemia effects. Further study may provide insights into the mechanisms involved in preventing leukemia development after allogeneic BMT and IL-2 in AKR/J mice.

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英文抄録の邦訳

T細胞性白血病好発系であるAKR/Jマウスに対し、H-2一致マウスよりの同種骨髄移植及び間欠的IL-2投与により、白血病発症を抑制しえた。同様にC3H/Heマウスより骨髄移植されたマウスでも、PBSを投与された群では、無処置AKR/Jマウスと同様の潜伏期間の後、T細胞性白血病を発症した。それに対して、IL-2を投与されたキメラマウスにおいては、40%のみしか白血病を発症しなかった。このIL-2による白血病発症抑制効果は、１年間のみIL-2を投与した群では認められなかったが、その原因は、持続的な抗白血病効果を長期間に亘り維持しえなかったことによると考えられた。IL-2投与群では、脾細胞のLAK及びNK活性が有意に亢進していた。AKR/J由来のT細胞リンパ腫のcell lineの細胞に対しても、IL-2投与群で細胞障害活性の亢進が認められた。また、LPS,PWM,IL-2に対する脾細胞の反応性も、IL-2投与群で亢進していた。脾細胞のTNF-alpha産生能も、IL-2投与群で増強していた。PCR法によるmRNAの解析では、IL-2投与キメラマウスの脾細胞において、IL-1beta,IFN-gamma,TNF-alphaの表出が増強していた。白血病発症後のキメラマウスから取り出した白血病細胞の性格は、IL-2反応性の点では、IL-2 投与による影響は認められなかった。これらの実験結果より、AKR/Jマウスにおいて同種骨髄移植とIL-2 投与によりT細胞性白血病の自然発症が部分的に抑制されるのは、GVL効果の亢進に起因すると考えられた。しかし、その抗白血病効果の詳細な機序の解析には、さらなる実験データの積み重ねが必要と考えられた。

Introduction

It is known that AKR/J mice are programmed to develop spontaneous thymic lymphoma / leukemia through sequential steps that are under the control of genetically determined factors[1].There are several reports supporting the concepts that leukemic transformation in AKR/J mice occurs in hematopoietic stem cells[2], and that T lymphocytes are the final targets of leukemogenesis by murine leukemia virus[3-5]. The thymus is considered to play a major role in the development of the disease. Thymectomy at the age of 1 - 3 months prevents T cell lymphoma / leukemia development in AKR/J mice[6]. Pollard et al. showed that allogeneic bone marrow transplantation (BMT) from H-2-incompatible mice (DBA/2) that have genetic resistance, introduced the capacity to resist the spontaneous development of leukemia in AKR/J mice[7]. Wustrow et al. showed the same prevention of spontaneous AKR/J leukemogenesis by allogeneic BMT from H-2-incompatible C57BL/6 mice[8]. Similarly, Tanaka et al. suggested that bone marrow (BM) cells from H-2-compatible CBA/H mice suppressed leukemogenesis and rendered the chimeras free of leukemia[9]. On the contrary, when AKR/J mice were transplanted from H-2-incompatible BALB/c mice, H-2-compatible C3H/Bi mice, or syngeneic young AKR/J mice, the chimeras developed spontaneous leukemia of the donor-origin within the same time-period as that in untreated controls[1, 7, 10].

An injection of some kind of nonlymphomagenic ectopic virus into young AKR/J mice (1 - 60 days old) inhibited spontaneous T cell lymphoma / leukemia development[11]. The virus seems to act through interference with the establishment of dual tropic virus in the thymus, thereby preventing potential leukemic cells to overt T cell lymphoma / leukemia. Also the elimination of potential leukemic cells with antibodies early after birth is reported to be useful for prevention of spontaneous AKR/J T cell leukemogenesis[12]. It is suggested

that prevention of lymphoma / leukemia development by passive antiviral immunotherapy involves elimination of potential leukemic cells representing the initial tumorigenic phase in AKR/J leukemogenesis.

More recently an immunotherapy with recombinant human interleukin-2 (IL-2) and some immunomodulations have been reported to lead to significant graft-versus-leukemia (GVL) effects in H-2-compatible allogeneic BMT settings as well as H-2-incompatible settings[13-16]. Thus, the present study was designed to examine whether a combination of IL-2 and BMT from C3H/HeJ mice that are a sensitive strain to leukemia could induce resistance against the development of spontaneous leukemia in AKR/J mice.

Materials and methods

Animals AKR/J (H-2^k,Thy1.1) and C3H/HeJ (H-2^k, Thy1.2) mice which were MHC-compatible but non-MHC-incompatible were obtained from Charles River Japan Inc. (Atsugi, Kanagawa, Japan) and Crea Inc. (Fuji, Shizuoka, Japan), respectively. The mice were raised under specific pathogen-free conditions in the animal facility of Hokkaido University School of Medicine. Six- to 8-wk-old male mice were used for both donors and recipients in BMT.

BMT Recipient AKR/J mice were irradiated with 860 cGy at a dose rate of approximately 70 rad / min from MBR-1520R X-irradiator (Hitachi Medical Co., Tokyo, Japan). BM cells of C3H/HeJ mice were collected by flushing of femurs and tibias with RPMI 1640 medium (RPMI) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). Within 6 hours after irradiation, recipient mice were injected with $1x10^7$ BM cells in 0.5 ml of RPMI via the lateral tail vein. All the recipients were given drinking water with antibiotics (minocycline 100ng / ml) for 3 weeks after BMT.

IL-2 therapy Recombinant IL-2 was kindly supplied by Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan). No endotoxin was detected in IL-2 (< 0.25 ng / ml). IL-2 regimen consisted of daily intraperitoneal injections of 5,000 units (in 0.1 ml of PBS) for 7 consecutive days a month. The therapy was started immediately after BMT on the first day of a month and continued for one year in one experimental group and forever in another group. Control chimeras received 0.1 ml of PBS in the same manner as above.

Preparation of cell suspension Spleen cells were removed aseptically from two or three donors of each group at 6 or 7 months after BMT and were crushed in RPMI with the piston of a glass syringe. The respective cell

suspensions were passed through a single layer of cotton gauze. For analysis of cytokine mRNA expressions, these cells were treated with Tris-NH_4Cl to lyse red blood cells. Leukemic cells from chimeras with overt leukemia were also prepared for IL-2 proliferation assay by the same method as above.

Assessment of chimerism Spleen cells from chimeras were analyzed for susceptibility to cytolysis by specific antibodies that recognized Thy1.2. Anti-Thy 1.2 monoclonal antibody was purchased from Meiji Institute of Health Science (Tokyo, Japan). Spleen cells were incubated with optimally diluted (x 50) antibodies for 30 min at 4°C followed by incubation with diluted (x 10) rabbit complement for 60 min at 37°C. The percentage of dead cells was evaluated by using the dye-exclusion method with 0.25% trypan blue. The cytotoxic index was determined according to the following formula : [(% of live cells in complement control) - (% of live cells in experiment) / (% of live cells in complement control)] x 100.

^{51}Chromium release assay The *in vitro* cytotoxic potential of spleen cells was evaluated in a 4-hour ^{51}chromium release assay against the LAK target P815, the NK target YAC1,and the AKR/J T cell lymhpoma cell line BW5147. The target cells were labeled with 3.7MBq of $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, MA, USA) in 1 ml of RPMI for 1 hour. The cells were washed three times with RPMI and plated in round-bottomed 96-well microtiter plates (Coster, Cambridge, MA, USA) at a concentration of $1x10^4$ cells / well. Various numbers of effector cells were added in a final volume of 200µl / well. Maximum isotope release was measured by the addition of 0.1 N HCl to the target cells ; spontaneous release was measured by the addition of RPMI alone. The plates were incubated for 4 hours at 37°C in a humidified atmosphere with 5% CO_2 in air. The culture supernatant was harvested and was counted in ARC-500 gamma counter (Aloka Co., Ltd., Tokyo, Japan).

The percentage specific lysis was calculated by the following formula : [(Experimental cpm - Spontaneous cpm) / (Maximum cpm - Spontaneous cpm)] x 100. All determinations were made in triplicate at various effector to target ratios. The data were calculated as mean ± SD.

Mitogen- and IL-2-induced responses Spleen cells were resuspended into RPMI supplemented with 10% heat-inactivated FCS (Biocell, Rancho Dominguez, CA, USA) and $5x10^{-5}$ M 2-mercaptoethanol. $5x10^5$ / 100 µl spleen cells were co-cultured with 10 µg / ml of concanavalin A (ConA) (Pharmacia Fine Chemicals, Uppsala, Sweden), 10 µg / ml of phytohemagglutinin (PHA) (IBF Biotechnics, Villeneuve-la-Garenne, France), 10 µg / ml of pokeweed mitogen (PWM) (Sigma Chemicals Co., St. Louis, MO, USA), 10 µg / ml of lipopolysaccharide (LPS) (Difco Laboratories, Detroit, MI, USA), or 1,000 units / ml of IL-2 in flat-bottomed 96-well microtiter plates (Costar, Cambdridge, MA, USA). All the cultures were set up in triplicate. Two days later, 37kBq of ^{3}H-TdR (New England Nuclear, Boston, MA, USA) was added to each well and after a 6 hour incubation period, each sample was harvested onto paper filters with a multi-sample harvester (Skatron, Lier, Norway). After the samples had been dried, they were applied to a liquid scintillation counter (Aloka Co., Ltd., Tokyo, Japan). The stimulation index was calculated as follows : ^{3}H-TdR incorporation of spleen cells stimulated with mitogen or cytokine / ^{3}H-TdR incorporation of unstimulated spleen cells.

To determine whether the IL-2 administration changed the characteristics of leukemic cells and IL-2 stimulated the proliferation of these cells, IL-2 responses of leukemic cells from each chimera were tested by almost the same method as above. Only the cultured cell number was decreased to 1 x 10^4 / 100 µl.

TNF-alpha production of spleen cells For the TNF-alpha assay, $5x10^6$ spleen cells were cultured with 10 μg of LPS in flat-bottomed 24-well culture plates (Becton Dickinson Labware, NJ, USA). On the next day, the culture supernatants were collected as the TNF-alpha source. $1x10^4$ TNF-alpha sensitive LM cells were cultured in 200 μl medium in the presence of the samples for 48 hours and 10 μl of MTT solution was added for the final 6 hours. Each sample was stained by acid-isopropanol (100 μl) and the plates were read on MTP - 100 microplate reader (Corona electric, Katsuta, Ibaragi, Japan) with a test wavelength of 570 nm and a reference wavelength of 630 nm.

mRNA expression for cytokines In the present study, the expression of IL-1 beta , IL-2, IL-4, IL-6, TNF- alpha and IFN- gamma mRNA in spleen cells were investigated using the reverse transcriptase - polymerase chain reaction (RT-PCR). The total RNA was extracted from the cells using guanidine thiocyanate / phenol / chloroform. Each 5 μg of RNA was reverse-transcripted by 600U murine Molony leukemia virus reverse transcriptase (BRL, Grand Island, NY, USA) with 150 pmol of random hexamer, and 1 / 20 th of the resulting cDNA was used for semiquantitative PCR.

The primers (IL-1 beta A 5'ATTAGACAGCTGCACTACAGGCTC3',
IL-1 bata B 5'AGATTCCATGGTGAAGTCAATTAT3' [17],
IL-2 A 5'ACATTGACACTTGTGCTCCTTGTC3',
IL-2 B 5'TTGAGGGCTTGTTGAGATGATGCT3' [18],
IL-4 A 5'AGTTAGTTGTCATCCTGCTCTTCT3',
IL-4 B 5'CGAGTAATCCATTTGCATGATGCT3' [19],
IL-6 A 5'GTCTATACCACTTCACAAGTCGGA3',
IL-6 B 5'TTGGATGGTCTTGGTCCTTAGCCA3' [20],
IFN-gamma A 5'CACGGCACAGTCATTGAAAGCCTA3',
IFN-gamma B 5'TGAGGCTGGATTCCGGCAACAGCT3' [21],

TNF-alpha A 5'ACCCTCACACTCAGATCATCTTCT3',
TNF-alpha B 5'CAGATTGACCTCAGCGCTGAGTTG3' [22],
beta-actin A 5'AGGGAAATCCTGCGTGACATCAAA3',
beta-actin B 5'ACTCATCGTACTCCTGCTTGCTGA3' [23]) were synthesized on a 380B DNA synthesizer (Applied Biosystems, Inc, Foster City, CA, USA). A volume of 5 μl of cDNA was added to the reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM deoxynucleotide triphosphates, 100 pmol of each primer, and 2.5 U Taq polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). Each 100 μl of the sample was overlayed with 50 μl of mineral oil and incubated in a thermal cycler for 30 sec at 94°C, 30 sec at 55°C, and 2 min at 72°C. After 25 -, 30 -, and 35 - cycle amplification, each 7 μl of the sample was removed and analyzed by gel electrophoresis through 2% agarose gel containing ethidium bromide.

Monitoring of survival Each group consisted of 10 mice. The mice were checked daily to determine the data of death. Autopsy was also performed to examine the presence or absence of leukemia. Survival curves were computed by the method of Kaplan and Meier[24].

Statistical analysis *P* values for comparison of results in various groups were determined by Student's t-test. Survival data are based on the generalized Wilcoxon test[25].

Results

Chimerism of spleen cells In Table 1, the Thy1.2 positive spleen cells from representative mice of each group are shown. Six months following BMT, the analyses of Thy1.2 antigen on the spleen cells from chimeras showed a lower donor-type chimerism compared with C3H/HeJ mice. No clinical evidence of graft-versus-host (GVH) reaction was encountered in both PBS- and IL-2-treated chimeras.

Effect of IL-2 on cytotoxic activity Cytotoxic activities of isolated spleen cells were tested. Figure 1 shows the results at E:T ratios of 50:1. IL-2-treated mice have more enhanced cytotoxic activities against the LAK target P815, the NK target YAC1, and the AKR/J T cell lymphoma cell line BW5147 with statistically significant differences. Although the figure is shown with only one point of E:T ratio, the data at other E:T ratios showed a similar tendency.

Mitogen- and IL-2-induced responses of spleen cells PWM-, LPS-, and IL-2-stimulated responses of spleen cells from the IL-2-treated chimeras were higher than those of spleen cells from the PBS-treated chimeras (Fig 2). Furthermore, ConA- and PHA-stimulated responses also had a slight increase in spleen cells from the IL-2 treated chimeras, although, there were no statistically significant differences.

IL-2 responsiveness of leukemic cells from chimeras Normal spleen cells from AKR/J mice showed increased IL-2 responsiveness which were dependent on IL-2 concentrations in a cultured medium. On the other hand, IL-2 responsiveness of spleen cells from chimeras with overt leukemia did not respond to IL-2, and also there were no differences of IL-2 responsiveness in each group (Fig 3).

TNF-alpha secretion by spleen cells Spleen cells from IL-2 treated mice produced increased quantities of TNF-alpha after stimulation by LPS. The TNF-alpha content of spleen cell supernatants rose with a statistically significant difference (Fig 4). This materials were bioactive on the LM assay.

Cytokine mRNA expressions in spleen cells IL-1-beta and TNF-alpha mRNA expressions were detected from spleen cells of IL-2-treated mice after 25-cycle amplification, and were not detected from PBS-treated mice (Fig 5). IFN-gamma mRNA expression was not detected in both groups after 25-cycle amplification, although, it was clearly detected in the IL-2-treated mice after 30-cycle amplification. However, there were no enhancement of mRNAs for IL-2, IL-4 and IL-6 in the IL-2-treated chimeras.

Therapeutic antileukemic effects of IL-2 after allogeneic BMT Figure 6 shows the effects of IL-2 after allogeneic BMT on survival. All mice in the PBS-treated group died of leukemia, however, six mice in the IL-2 treated group which survived more than 450 days after BMT died without any evidence of leukemia. Allogeneic BMT with intermittent IL-2 treatment significantly improved the survival over the control group ($p < 0.05$), and achieved a prevention of leukemic development in 60% of the mice. On the contrary, the survival of the mice receiving IL-2 for only 1 year after BMT was not prolonged compared with that in the PBS-treated mice with no statistical significant difference (Fig. 7). All mice in both groups receiving IL-2 or PBS for 1 year died of leukemia.

Discussion

AKR/J mice display a high incidence of spontaneous T cell lymphoma / leukemia that arises predominantly in the thymus at age of 6-12 months[6], and a number of factors are involved in the high susceptibility of AKR/J mice to leukemia development. In previous studies, several methods to prevent spontaneous leukemia in AKR/J mice have been investigated. AKR/J mice were protected from developing the disease by thymectomy at the age of 1-3 months[6], allogeneic BMT from the mice that possess resistant genes for leukemia[7-9], injection of some kind of nonlymphomagenic ecotropic virus into young AKR/J mice (1-60 days old)[11], and elimination of potential leukemic cells with antibodies early after birth[12].

More recently an immunotherapy with IL-2 and H-2-incompatible allogeneic BMT has been reported to lead to a significant antitumor effect in leukemia-bearing mice[13, 14]. These reports suggest that allogeneic lymphocytes may be spontaneously alloactivated *in vivo* to induce potent GVL effects that are usually complicated by GVHD and may be further activated by a relatively low dose IL-2 administration *in vivo* after an initial induction of minimal residual disease by high dose chemoradiotherapy followed by BMT. Also it was revealed that GVL effect in MHC-compatible allogeneic BMT mice was enhanced by some immunomodulations[15, 16]. In such a H-2-compatible BMT setting, when donor BM cells were treated with anti - T cell antibodies plus complement prior to BMT, a beneficial GVL effect was completely cancelled.

In the syngeneic BMT settings, immunotherapies with IL-2 after BMT may be expected as a new immunotherapeutic tool for decreasing the relapse rate after BMT for hematological malignancies[26-28]. However, the mechanism of the antitumor effects in these treatments has not been extensively investigated. On the other hand, in the human settings, several researchers have shown that the administration of IL-2 after autologous BMT may result in

improved relapse-free survival, and that enhanced cytotoxic activities and *in vivo* induction of IFN-gamma and TNF-alpha may be the major antileukemic mechanism[29-34].

Thus, the present study was designed to investigate whether a combination of IL-2 and allogeneic BMT from H-2-compatible C3H/HeJ mice that are a sensitive strain to leukemia could induce resistance against the development of spontaneous leukemia in AKR/J mice, and to establish the major factors in antileukemic effects. It is important to analyze the efficacy of BMT from H-2-compatible C3H/HeJ, since there is the same genetical background in both donors and recipients as human HLA-identical and MLC-negative BMT.

As shown in Table 1, chimeras were reconstituted by the cells derived from both donors and recipients. The data for this study suggested the possibility for the existence of mixed chimerism, a decreased number of T cells, or low Thy1.2 antigen expression in some chimeras. In terms of mixed chimerism, there are many mixed chimerism status in recipients after human allogeneic BMT. Therefore, it is rather useful to apply the experimental data for human BMT settings, and IL-2 administration may be applicable with human BMT as well. Antileukemic effects observed in the present study appeared to be caused by donor-derived cells partially, resulting in so called GVL effects in allogeneic BMT settings.

Biological stimulation of the immune system in IL-2-treated chimeras was clear. LAK (anti - P815) and NK (anti - YAC1) cytotoxic effector function of spleen cells were both significantly increased. These activated spleen cells also mediated the antileukemic effector function as demonstrated by the effect of spleen cells on the cytotoxicity against BW5147 cells (T cell lymphoma cell line of AKR/J origin). These changes are usually observed after autologous BMT and IL-2 treatments in clinical settings, and these non-MHC-restricted killer activities are thought to be the significant effector cells to eliminate minimal residual disease[29-33]. In the present study, these non-specific

cytotoxicities seem to be useful in the prevention of spontaneous leukemia development, and may be the major factors in GVL effects.

Similarly, mitogen responses from IL-2-treated mice were increased in comparison to PBS-treated mice. Interestingly, the enhancement was seen in not only T and B-cell-dependent mitogen response (PWM) but also B-cell-dependent mitogen response (LPS). These data suggest that the administration of IL-2 markedly evokes the immune system and indirectly stimulates the B-cell system as well as the T-cell system. On the contrary, ConA- and PHA- responses were not enhanced in the IL-2 treated chimeras.

Also the enhanced IL-2 responsiveness in the IL-2-treated mice seems to induce immunological activation via the cytokine network. The reasons why the enhanced IL-2 responsiveness did not induce the higher ConA- and PHA-responses can not be sufficiently understood from these experiments. One of the reasons may be related to the decreased T cell population observed in the chimerism assay. The modified expressions of IL-2 receptors may also explain the fact.

The IL-2 responsiveness of leukemic cells were not detected, and there was no difference between the goups. Therefore, IL-2 administration did not change the character of leukemic cells in the aspects of IL-2 responsiveness. The fact that the leukemic cells have lost the IL-2 responsiveness after overt leukemia development may suggest the change of the IL-2 receptor for it's affinities. However, it is not yet clear whether this change may be related to leukemogenesis.

It is known that IL-2 induces secretion of TNF-alpha, IFN-gamma, IL-3, IL-5, IL-6, GM-CSF, and M-CSF [34-36]. On the other hand, it is also known that the onset of GVHD is related to TNF-alpha and IFN-gamma[37, 38]. Recently, IL-6 has been revealed to be one of the key cytokines in GVHD[39]. However, it is not clear how soluble effector molecules are involved in GVL. Therefore, I investigated mRNAs for some cytokines in spleen cells and studied the

relationship between the cytokines and antileukemic potentials in the IL-2-treated mice. The reason why I did not measure the serum cytokine concentrations according to the assay of ELISA etc., is because the method of detection for mRNA expressions is more sensitive than that by the above. Therefore, the PCR technique is suitable for screening. Additionally, it was reported that the rise in serum cytokine concentrations corresponded with the appearance of mRNA in circulating mononuclear cells[35]. The inceased mRNA expressions were detected for IL-1 beta, TNF-alpha, and IFN-gamma in the IL-2-treated mice. These findings indicated that the IL-2 induced activation of the immune system was complicated and that there were more cytokines related to GVL than previously reported. Recently, Takikawa et al. reported an synergistic antitumor effect between IFN-gamma and IL-1 alpha/beta in the rejection of allografted tumor cells[40]. Therefore, the enhanced expressions of these cytokines mRNA observed in this experimental model may indicate that these cytokines play important roles in GVL. In the present study, TNF-alpha producing capacity of spleen cells was also enhanced in the IL-2-treated mice, implying TNF-alpha was probably one of the most important effector cytokines inducing GVL effects. However, the strict evaluation of the relationship between the cytokines and GVL effects in this experimental model awaits further investigation.

The survival curves in both experiments show that IL-2 administration after H-2-compatible allogeneic BMT is useful for preventing spontaneous leukemia development in AKR/J mice, and that IL-2 must be administered for a long term to prevent later leukemogenesis. It may be that the IL-2 activated immune system in chimeras only prolongs the onset of leukemia development, and it is not possible to completely eradicate the potential leukemic cells by this treatment schedule. However, the fact that there were 60% of IL-2-treated chimeras with non-leukemic death after more than 450 days post BMT suggests that there is a possibility to preventing spontaneous

leukemia development completely by some modified treatment schedules. These data may indicate the relationship between the suscetibility to leukemogenesis and aging. Further studies should be performed to determine the exact mechanism for the antileukemic effects induced by IL-2 after H-2-compatible BMT, and the best schedule of IL-2 administration to prevent the development of spontaneous leukemia in AKR/J mice. This analysis would contribute to a more useful and effective therapeutic protocol for patients with leukemia, especially for patients with T cell leukemia.

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Table 1 Chimerism in spleen cells following allogeneic BMT

Animals	Thy1.2 (mean ±SD)
AKR/J (Thy1.1)	0.97 ± 1.67
C3H/HeJ (Thy1.2)	17.75 ± 6.58
Chimeras (AKR/J ← C3H/HeJ)	8.33 ± 1.40

Spleen cells were analyzed when normal mice and PBS-treated chimeras were 6 mo old. The cytotoxic index (percentage) against Thy1.2 antigen on spleen cells is shown.

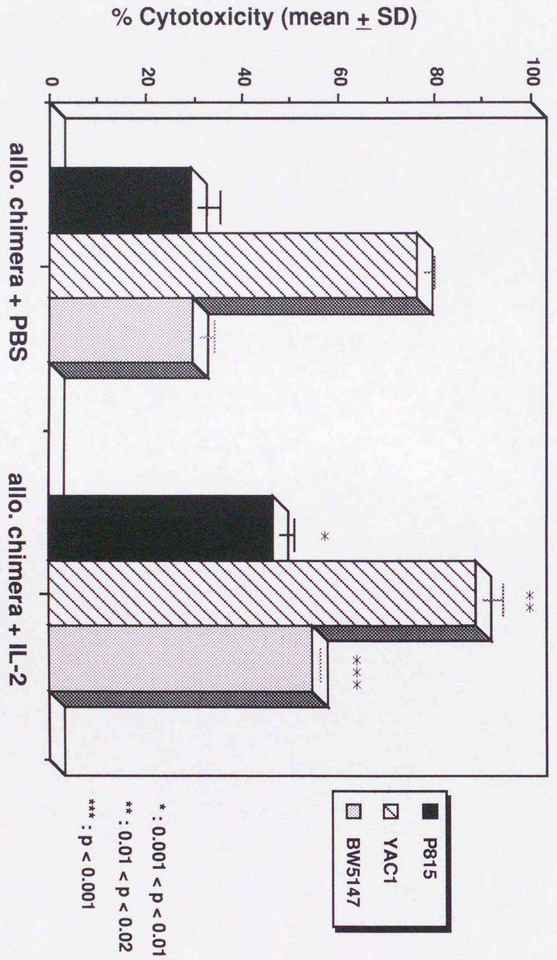

Fig. 1

Fig. 1. Effects of IL-2 therapy on cytotoxic activities of spleen cells. Spleen cells were assessed for lysis of P815, YAC1, and BW5147. Data shown are mean percent lysis ± SD at an E:T ratio of 50:1.

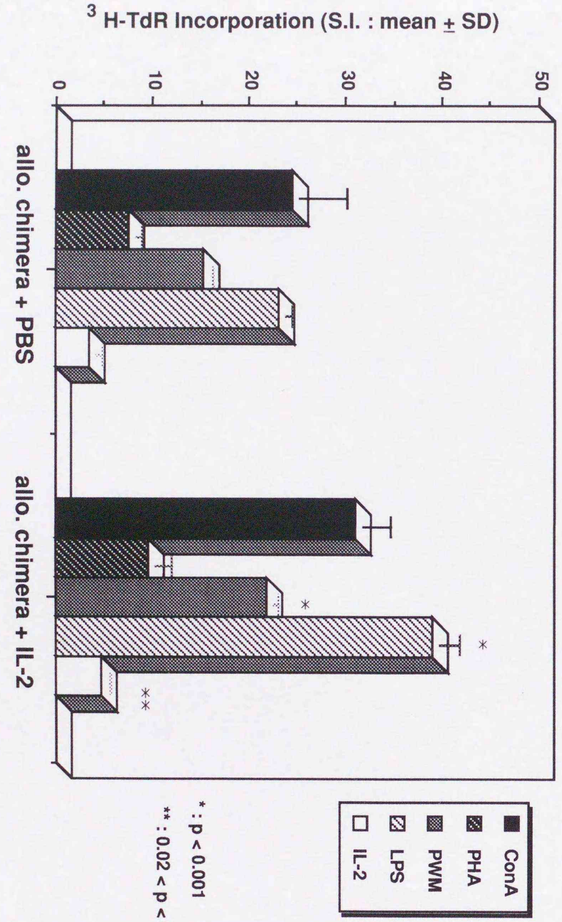

Fig. 2

Fig.2. Mitogen and IL-2 responses of spleen cells from each chimera. Spleen cells were co-cultured with ConA, PHA, PWM, LPS, or IL-2. Data shown are the stimulation index of mean ^{3}H-TdR incorporation ± SD.

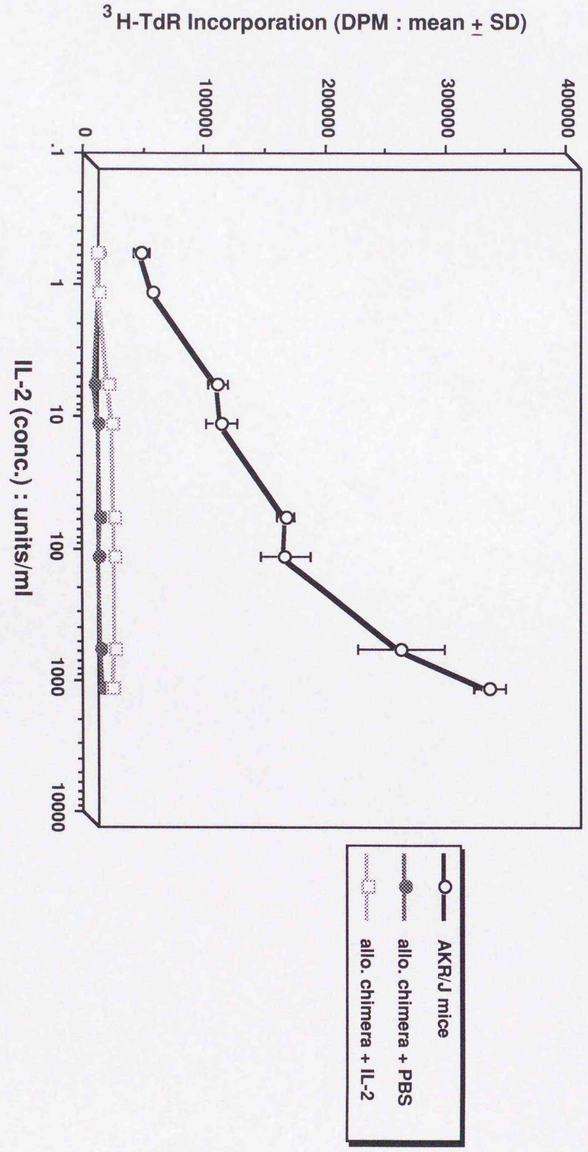

Fig. 3

Fig. 3. IL-2 responsiveness of spleen cells from normal AKR/J mice or chimeras with overt leukemia. Spleen cells from normal AKR/J mice were positive control for IL-2 responsiveness. Leukemic cells from chimeras with overt leukemia were obtained 1 year after BMT. Data shown are mean ^{3}H-TdR incorporation (cpm) ± SD.

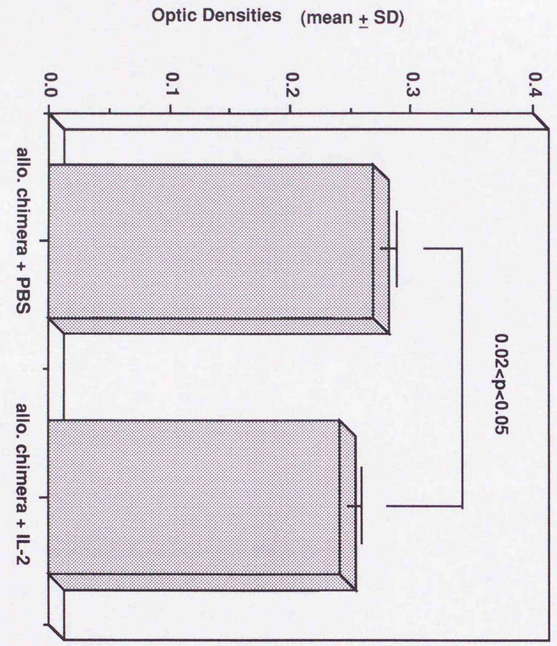

Fig. 4 31

Fig.4. LPS-stimulated TNF secretion from spleen cells. Spleen cells were obtained from chimeras and cultured as described in Materials and Methods section. Data shown represent mean optic densities and ± SD.

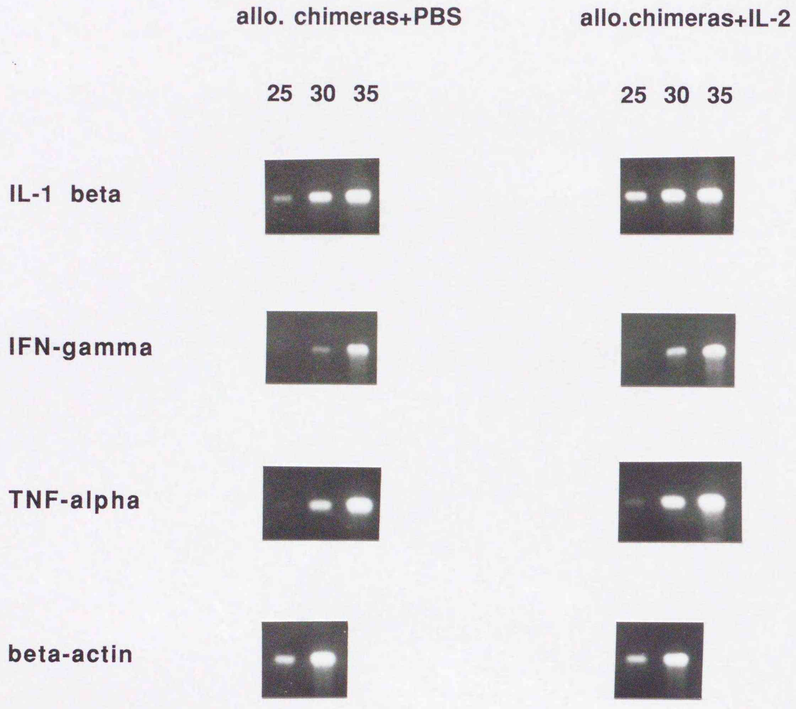

Fig. 5

Fig. 5. Analysis of mRNA expressions using semiquantitative polymerase chain reaction. Total RNA was isolated from spleen cells. Isolated total RNAs were reverse transcripted into cDNA, and amplified by PCR (25, 30, and 35 cycles) with primer for IL-1 beta, IFN-gamma, TNF-alpha, and beta-actin. Amplified IL-1 beta, IFN-gamma, TNF-alpha, and beta-actin mRNAs were 441, 477, 423, and 478 bae pairs, respectively.

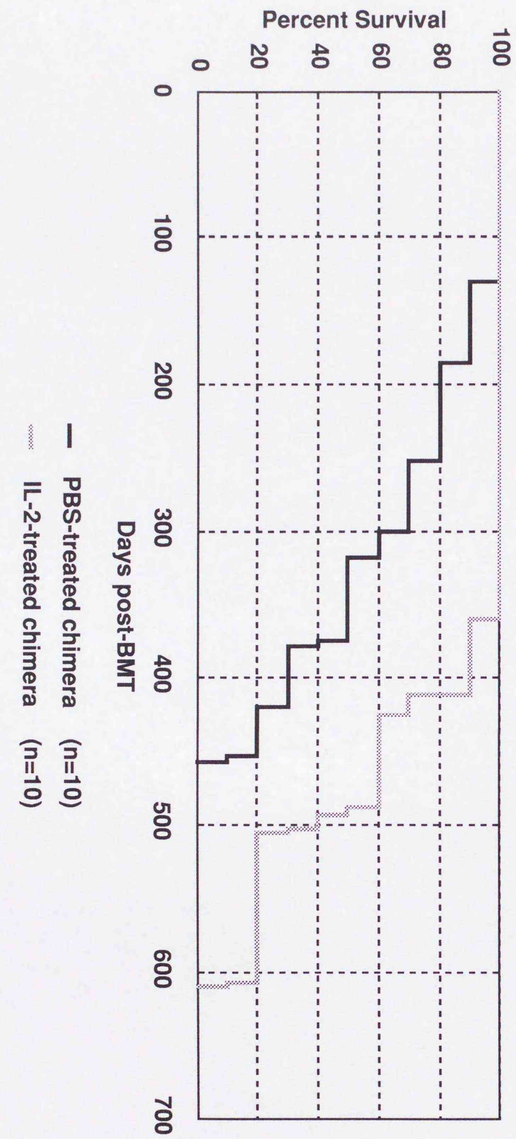

Fig. 6

Fig. 6. Pattern of survival in the IL-2 or PBS therapy after allogeneic BMT. IL-2 or PBS injection was started immediately after BMT and continued to the death. Mice were followed for survival to the death. p : PBS-treated group versus IL-2-treated group, < 0.05.

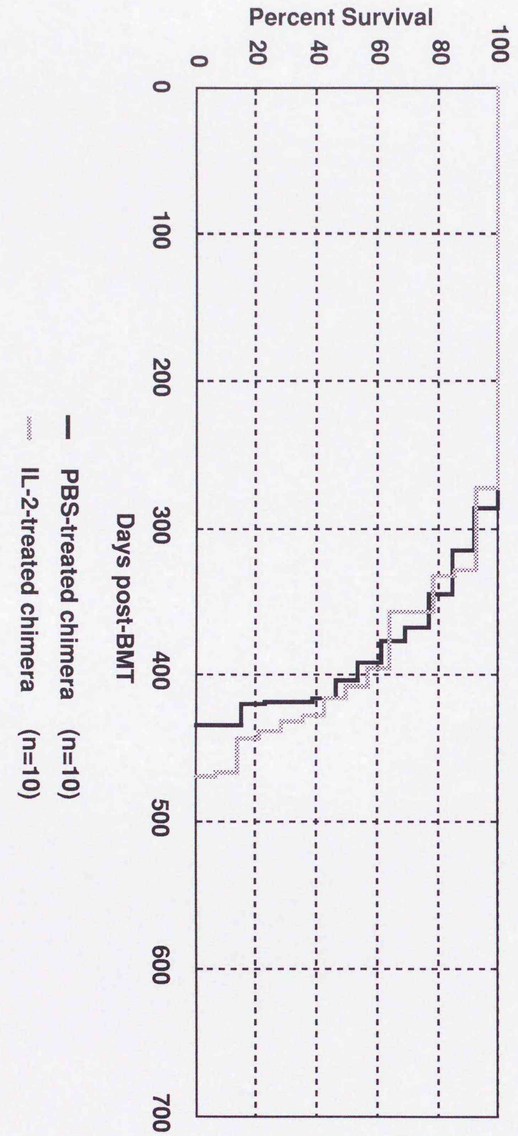

Fig. 7 37

Fig. 7. Pattern of survival in the IL-2 or PBS therapy after allogeneic BMT. IL-2 or PBS injection was started immediately after BMT and continued for 1 year. Mice were followed for survival to the death.