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Damage of radioprotection and antitumor effects of *Enterococcus Facalis* 2001

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Abstract

Some natural products are able to inhibit radiation effects and exert an antitumor effect with fewer adverse reactions; however, their antitumor effects are less than those of widely-used synthetic drugs. *Enterococcus Facalis* 2001 is a natural material that has been attracting attention, and we extracted this material with water and investigated the effect of continuous *Enterococcus Facalis* 2001 administration on radioactivity-induced reduction of hemocytes, in addition to the antioxidant and antitumor effects of *Enterococcus Facalis* 2001. Following a 1-week adjustment period, *Enterococcus Facalis* 2001 was administered intraperitoneally to male ICR mice at a dose of 500 mg/kg every other day for 2 weeks. Following administration, 2 Gy whole-body irradiation was performed and the counts of leukocytes, lymphocytes, and granulocytes and monocytes in the peripheral blood were determined 1, 3, 7, 15 and 30 days after irradiation. These cells were considered since they are closely associated with immunity to radioactivity. In a second experiment, *Enterococcus Facalis* 2001 was similarly administered to the mice for 2 weeks after a 1-week adjustment period, and 2 Gy whole-body irradiation was performed. The antioxidant effects in hemocytes were then investigated using 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), a radical generator. In a third experiment, 1×10^6 Sarcoma-180 cells were inoculated into the right thigh of mice, which were divided into four groups: control, *Enterococcus Facalis* 2001-treated, 6 Gy irradiated and *Enterococcus Facalis* 2001-treated + 6 Gy irradiated groups, and changes in tumor size were measured for 20 days. Statistical analysis was conducted using ANOVA for multiple groups. In the three experiments, administration of *Enterococcus Facalis* 2001 inhibited the reduction of hemocytes caused by whole-body irradiation, showed antioxidant effects against radioactivity, and inhibited tumor growth, respectively. In conclusion, our data suggest that the antioxidant effect of *Enterococcus Facalis* 2001 inhibits hemocyte reduction caused by whole-body irradiation and enhances immunological inhibition of tumor growth.



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Abstract



- We studied an immunological enhancement effect, radiation protection effect and anticancer action for *Enterococcus Facalis 2001* which was quality of natural product.
- We provided an experimental group in total four groups. We used an SOD activity detection kit as an antioxidant study and tested it. We performed CD4, CD8 and CD16 analysis by flow-cytometry as analysis of T-lymphocyte. In addition, we studied activity of natural killer cell. In the SOD-like active measurement, a rise was seen in a *Enterococcus Facalis 2001* administrated group. In CD4, CD8 and CD16 measurement, remarkable increase of *Enterococcus Facalis 2001* administrated group was seen for control group.
- Antitumor effect revealed decrease a *Enterococcus Facalis 2001* administrated group. Therefore, T cell and B cell of the lymphocytes are increased by a work of various cytokine with activation of an immune cell by polysaccharides of *Enterococcus Facalis 2001*, and it is thought that we activate immune reaction.
- β -D- glucan is included in *Enterococcus Facalis 2001*, and it is thought that there is it for reasons of the one of the antioxidation ability rises by SOD method. A killer cell and increase of suppressor T cell are thought about in *Enterococcus Facalis 2001* in this study. Therefore, it is thought that there is anticancer action by *Enterococcus Facalis 2001* intake by activation of a factor about attack to cancer cell.
- Immune therapy will attract attention in treatment for cancer in future. Therefore, we think that we must do a search of the target which is specific for cancer in treatment for cancer and basic researches of immune therapy from the genetic side.

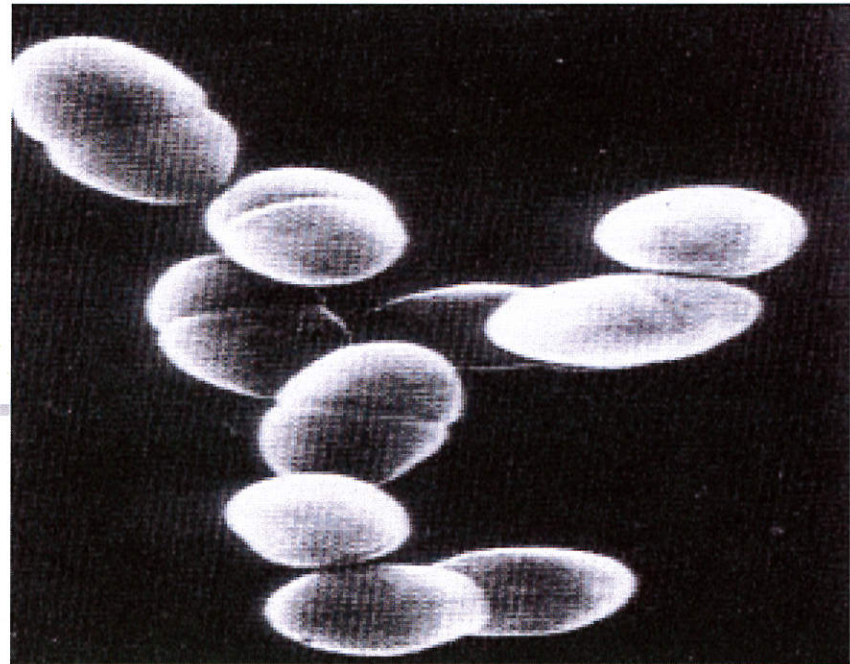
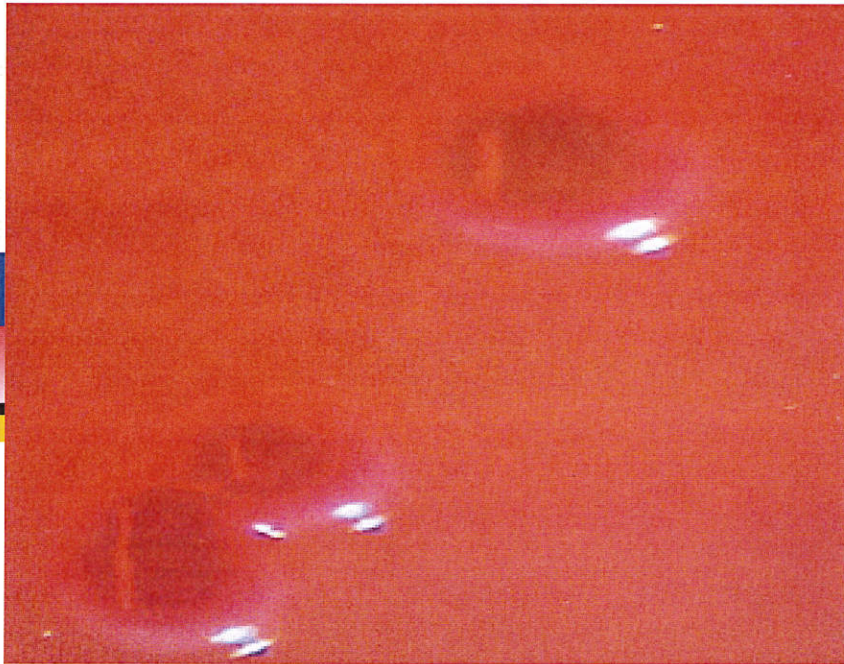
Purpose



Various effects were reported in *Enterococcus Facalis 2001*. We review an effect of *Enterococcus Facalis 2001* product in this study and are aimed at contributing to study in our data.

- ✦ Study of immunostimulation activity of *Enterococcus Facalis 2001*.
- ✦ Examination about a radiation protection effect by *Enterococcus Facalis 2001* with oral administration.
- ✦ Study about a mechanism of the anticancer effect by EF2001.
- ✦ It is reviewed reduction of a side effect by radiation protection at *Enterococcus Facalis 2001* with radiotherapy.

Enterococcus Faecalis



Enterococcus Faecalis under microscope (left) $\times 20$, (right) $\times 12900$

Material and Methods 1



The blood corpuscle measurement and T lymphocyte subsets analysis

- Study group:
- Control group (water)
- *Enterococcus Facalis 2001* of oral administration group
- 2Gy: Control(water)+2Gy group
- EF2001+2Gy: *Enterococcus Facalis 2001* of oral administration +2Gy group

Material and Methods 2



- Animal used for experiment; an ICR mouse (one five week of age ,♂, 6)
- Use machinery; radiation device (product made in *Philips Corporation*, MG226/4.5); CD4/CD8/CD3 3Color FCM, CD3/CD16 flow cytometry (product made in *BD company*) an erythrocytometer surveying instrument (*Nihon Kohden celltac- α*) automatically:
- Every day to the study end in o.p. at 500mg/kg medication method
- 2Gy radiation bath an irradiation condition administration two weeks later (0.355Gy/min)

Material and Methods 3



● 10 μ l collect blood of a caudal vein of a mouse, and drawing blood methods measure it with an automatic blood cell analysis.

● 3hp, 12hp, 1dp, 3dp, 7dp, 14dp and 28dp the measurement irradiation

● Measurement leukocytes: Leukocytes, lymphocytes, monocytes and granulocytes.

● Heart collects blood after administration 42nd, and T lymphocytes analysis, by whole blood method.

● Statistical analysis: Dunnett's test (5%, 1%)

Material and Methods 4



Anti-tumor effect

- Study group:
- Control group (water)
- *Enterococcus Faecalis* 2001 of oral administration group
- 6Gy: Control(water)+6Gy group
- *Enterococcus Faecalis* 2001 of oral administration+6Gy group

Material and Methods 5



- Animal used for experiment; C3H mouse (five weeks age, ♂, 8)
- Use machinery; radiation device (product made in *Philips Corporation*, MG226/4.5):
- Every day to the study end in o.p. at 500mg/kg medication method.
- Subcutaneous injection in 1×10^6 SCC-7 (Squamous Cell Carcinoma-7).
- Cancer injection: in a right femoral region

Material and Methods 6



● Local irradiation (0.341 Gy/min) to divide 6 Gy/ three times to the right hind leg an irradiation condition every other day from SCC-7 inoculation eight days

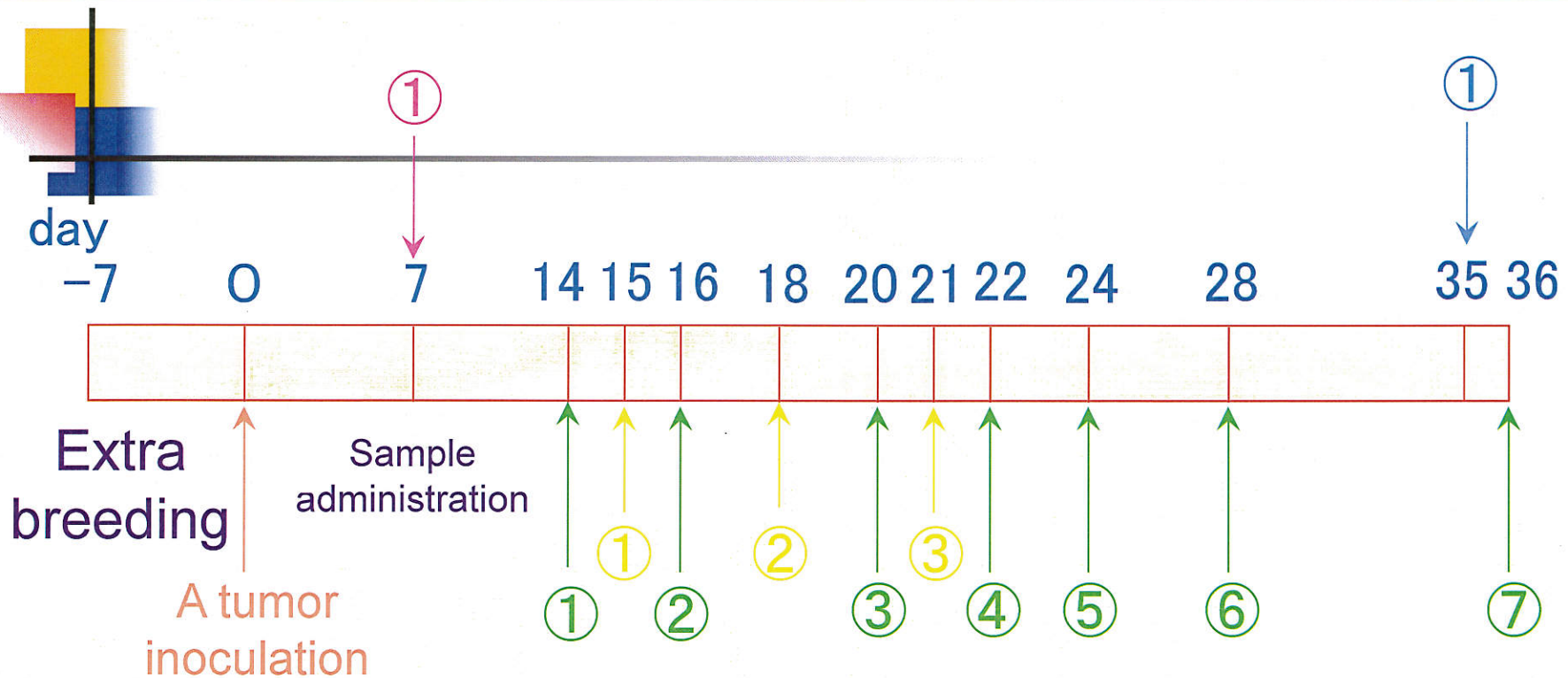
● The tumor size measurement measures a longer axis and a minor axis in vernier calipers from the back to enucleation of tumor after a tumor inoculation every 3rd on 8th

● Calculation methods of a tumor volume:

● Tumor volume (mm³) = $\frac{1}{2}$ (longer axis) \times (minor axis)²

● Statistical analysis; Dunnett's test (5%, 1%)

Experiment Schedule



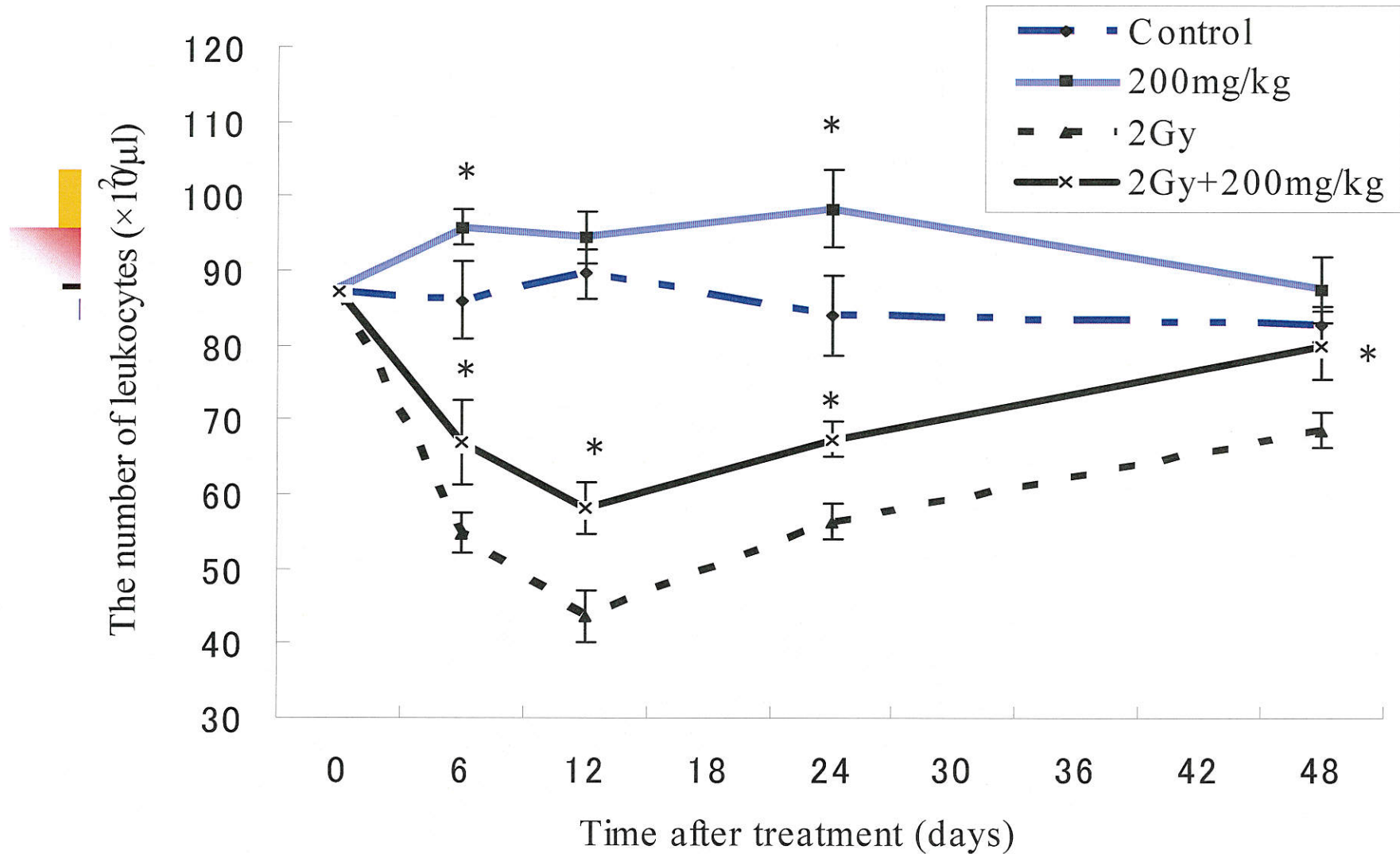
Measure every other day since tumor size measurement ---① 7th days

Enucleation of tumor ---①(35th days)

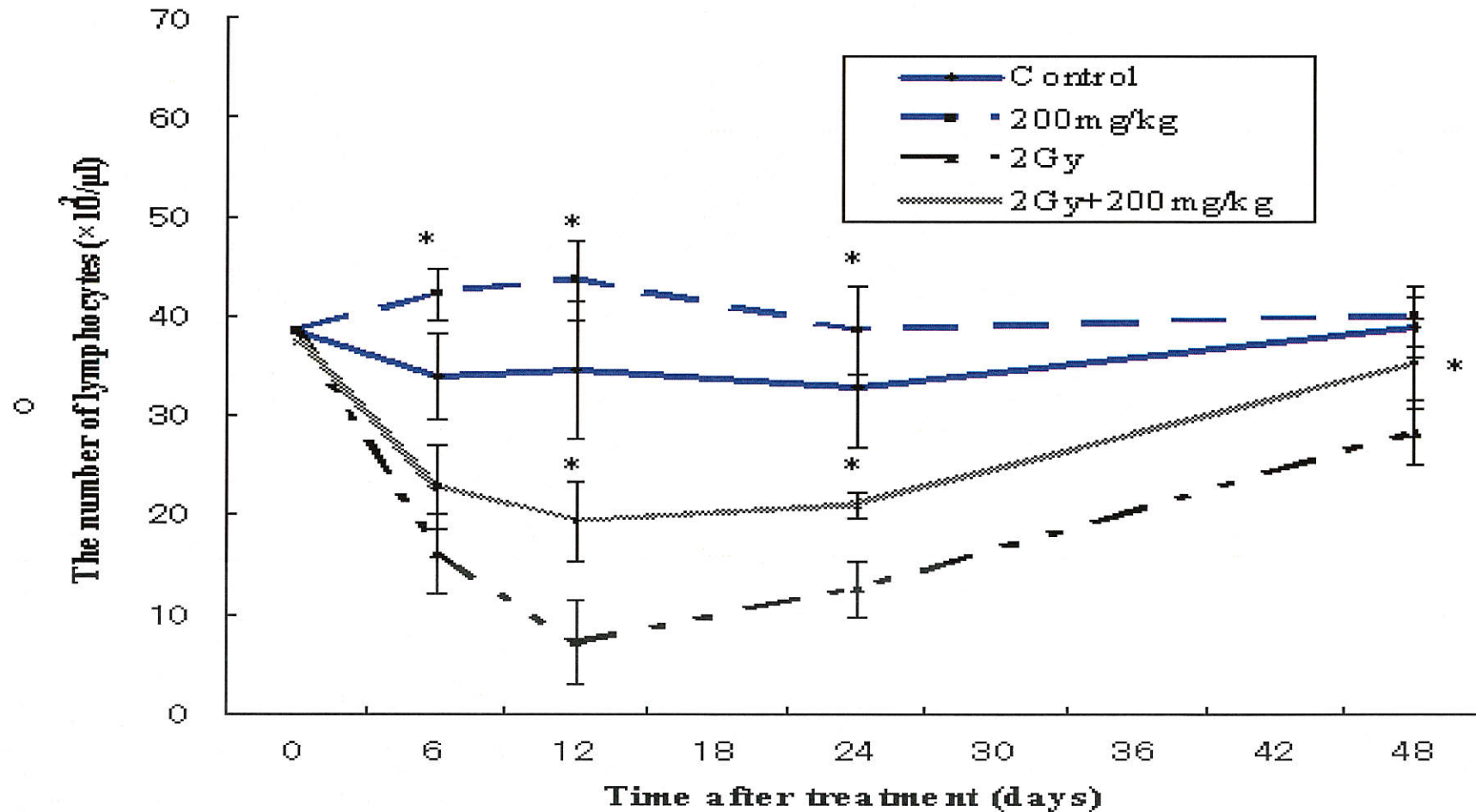
Roentgen radiation ---①-③(15,18,21 days judgment)

Blood count ---①-⑦(14,16,20,22,24,28,36 day judgment)

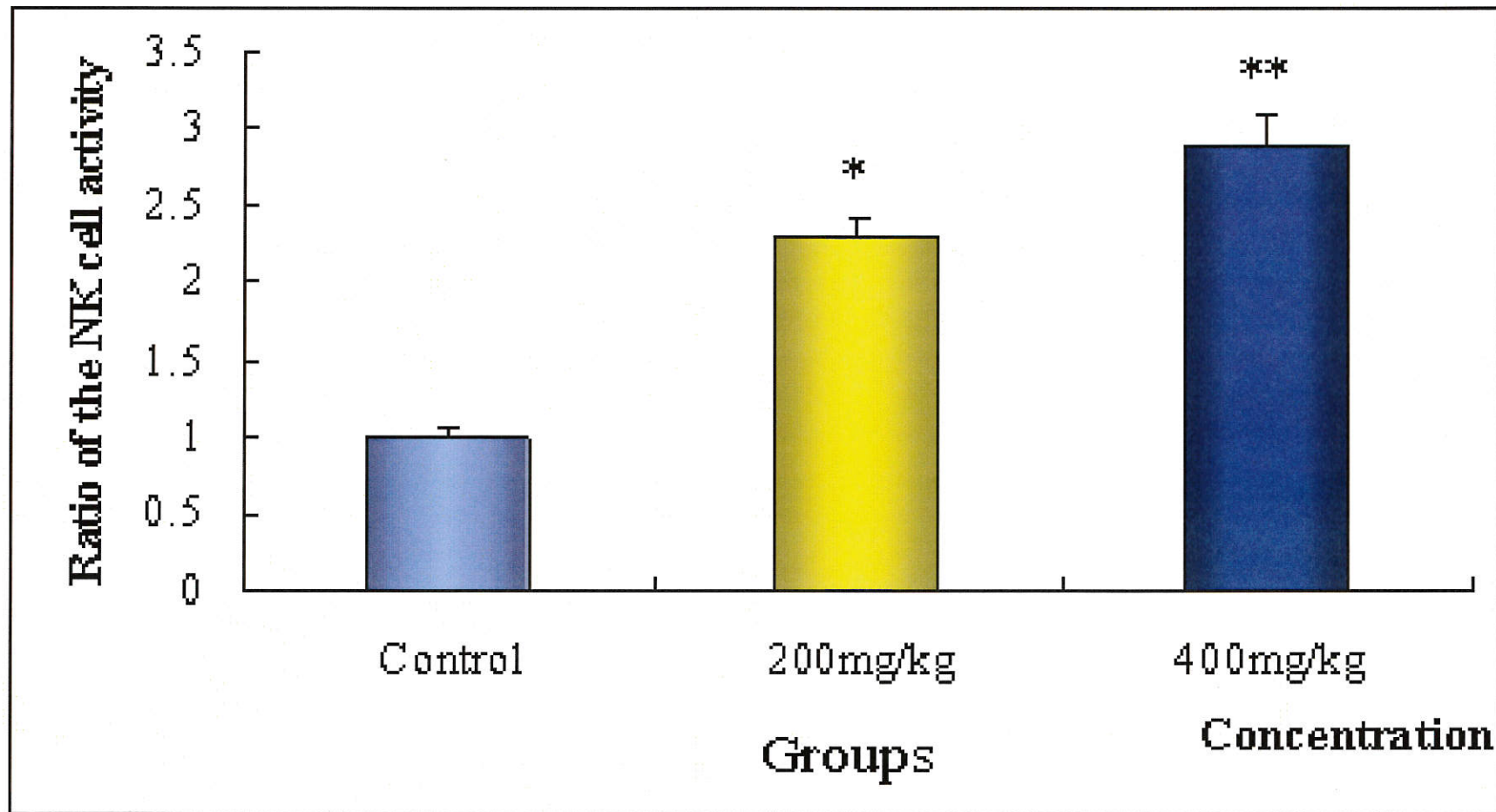
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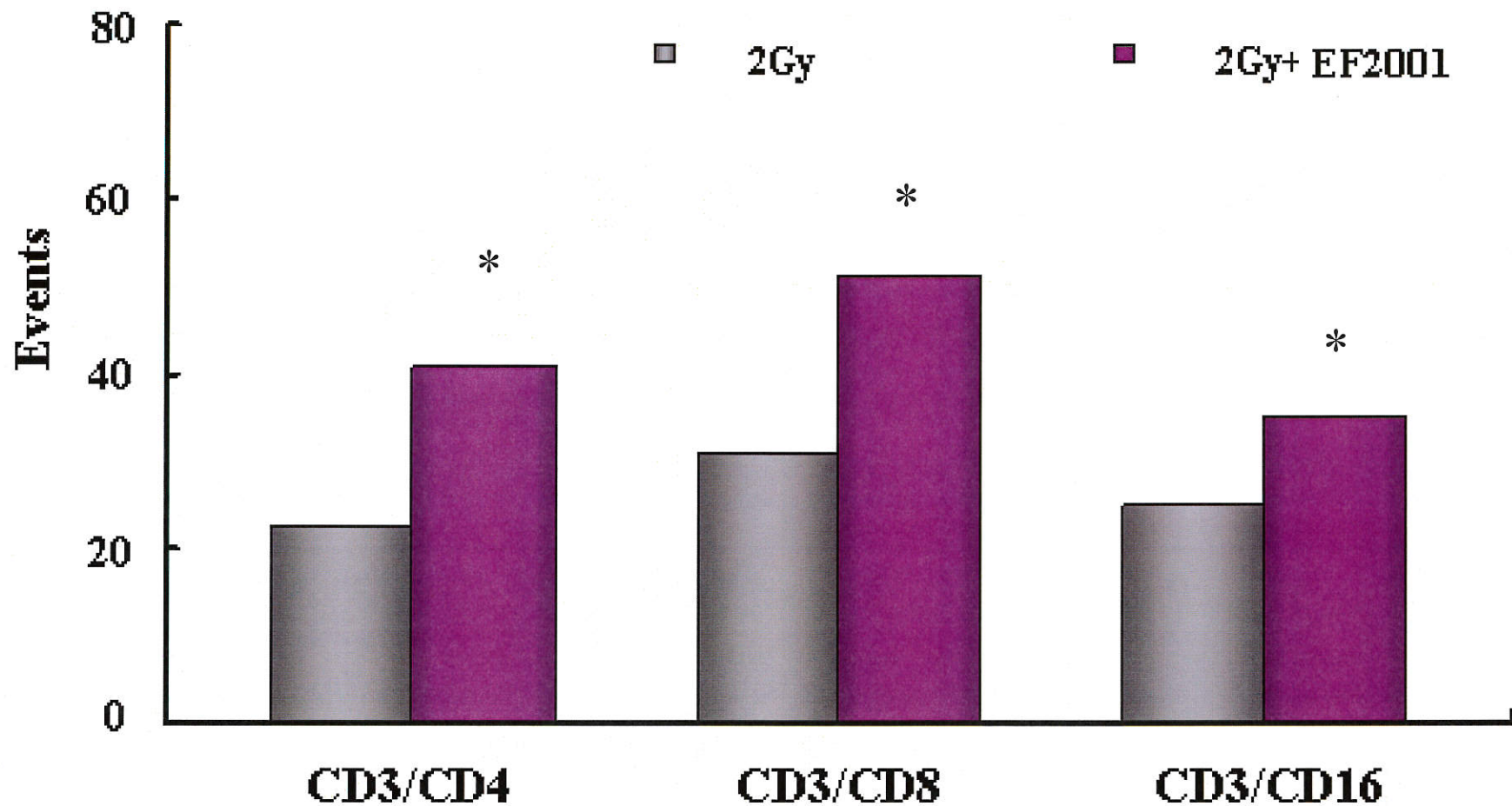
Leukocyte counts on different days after irradiation in mice of different groups. The number of leukocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. * Statistically significant ($P < 0.05$) from the control group.



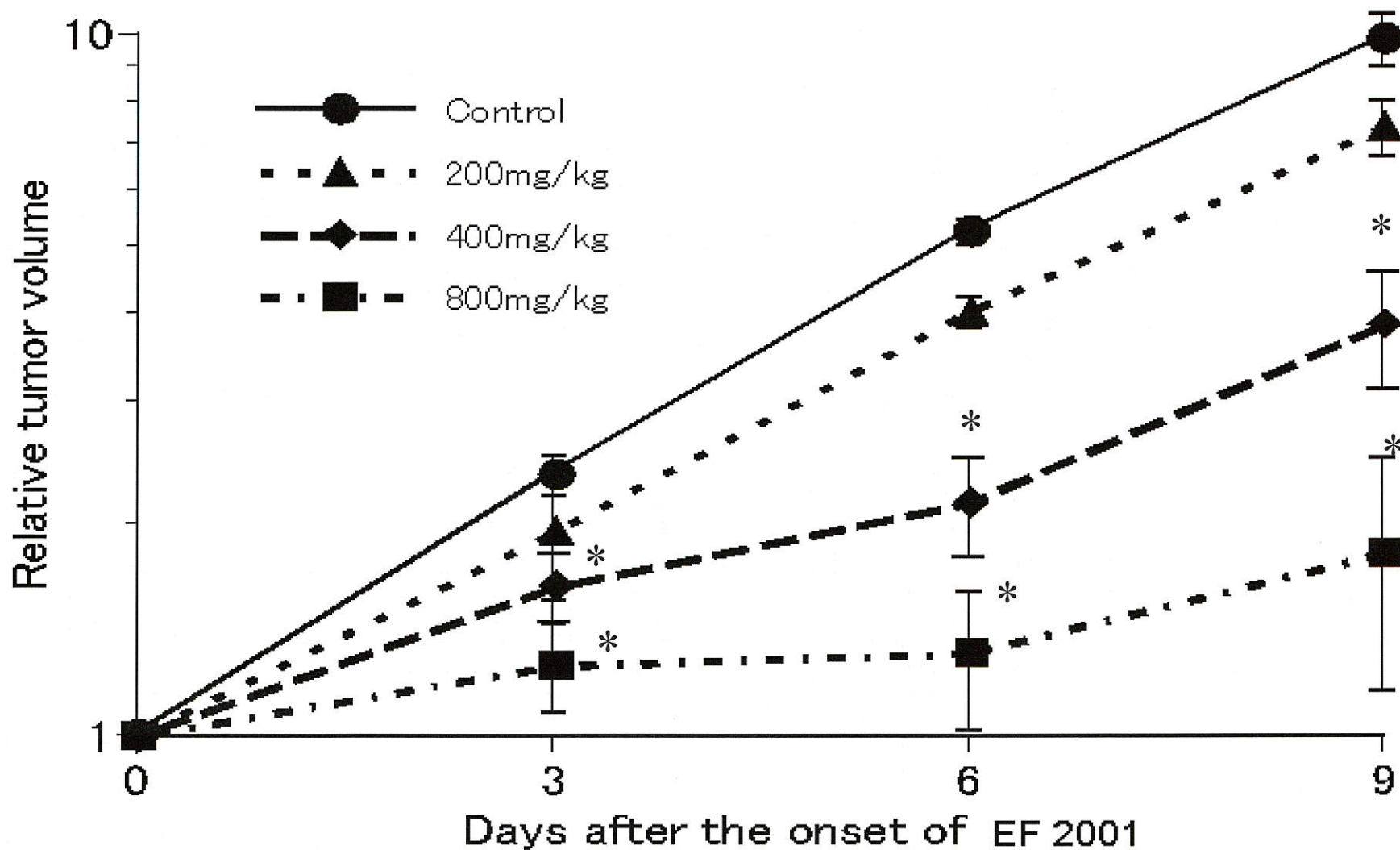
Lymphocyte counts on different days after irradiation in mice of different groups. The number of lymphocyte was calculated from the pre-irradiation values taken as 100%. The bars represent standard deviation. * Statistically significant ($P < 0.05$) from the control group.



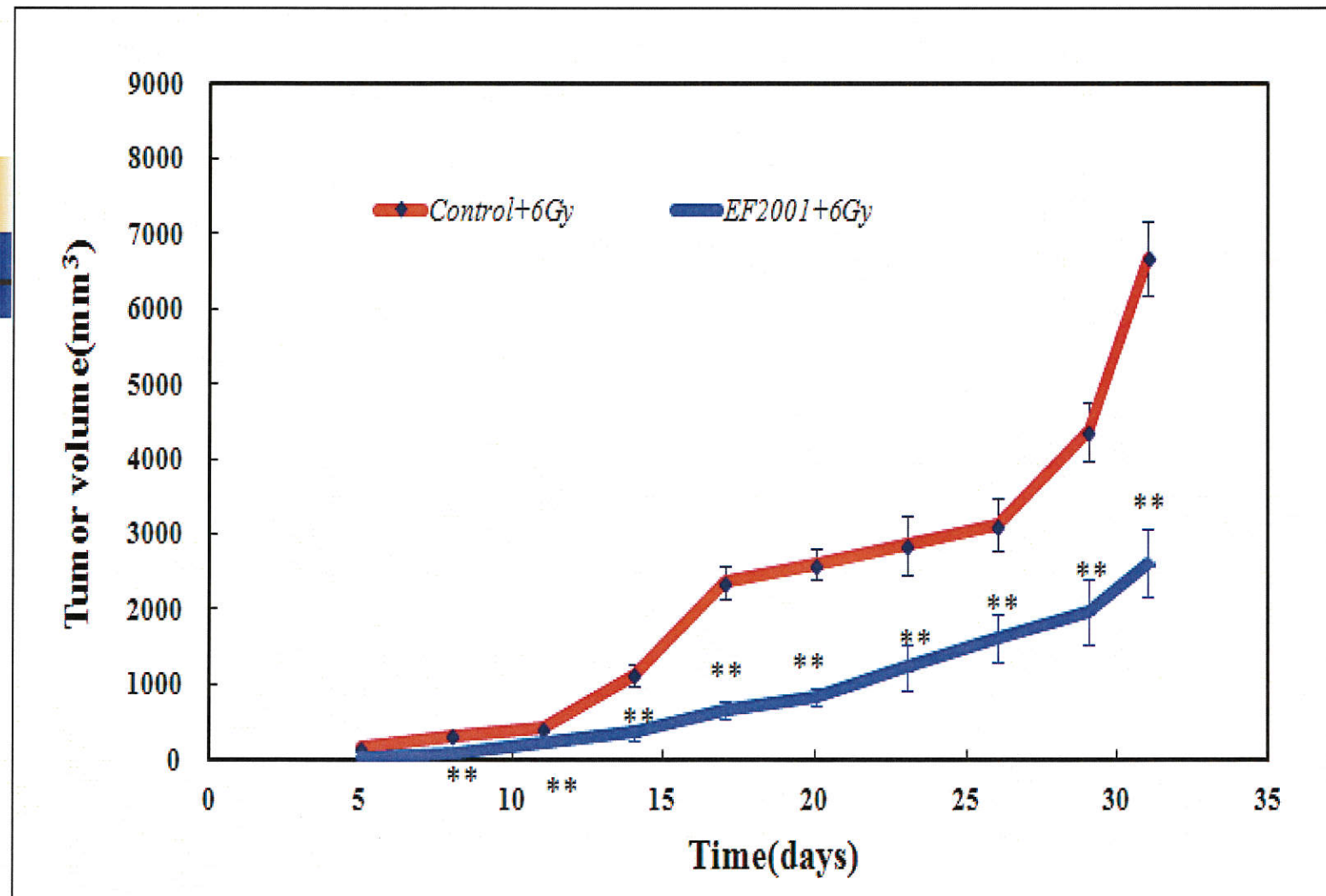
Repeated dose effect of EF 2001 on the NK activity in mice. Groups of ten mice each were subjected to each treatment. Results represent means \pm S.D. * Statistically significant ($P < 0.05$) from the control group.



The increased percentage of CD4+, CD8+ and CD16+ T-lymphocytes in PBLs compared to the experimental data baselines of the groups. The unit is in percentage (%). Significantly different from* $P < 0.05$ Control group vs. *EF2001* groups by Dunnett test.



Effect of EF2001 on the tumor growth in mice inoculated with SSC-7 carcinoma cells. Groups of ten mice each were subjected to each treatment. Results represent means \pm S.D. * Statistically significant ($P < 0.05$) from the control group.



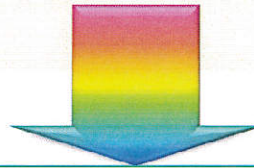
Effect of *Enterococcus Faecalis* 2001 on the tumor growth in mice inoculated with SCC-7 (Squamous Cell Carcinoma-7). Groups of ten mice each were subjected to each treatment. Results represent means \pm S.D. * Statistically significant ($P<0.05$) from the control group. ** Statistically significant ($P<0.01$) from the control group.



Discussion 1

The blood corpuscle measurement

- White blood cells, increase of lymphocytes counts were found by *Enterococcus Facalis 2001* administration of non-irradiation group.
- Decrease of a blood cell count was inhibited irradiation group by *Enterococcus Facalis 2001* administration, and meaningful early convalescence was found.



- The immunostimulation action that was strong in *Enterococcus Facalis 2001* was present.
- Helper T-cell is activated than the meaningful reinforcement of CD3/CD4, CD3/CD8, and CD3/CD16 and it is thought that NK-cell is activated.
- It is thought that a Radiation protection effect by *Enterococcus Facalis 2001* is the free radical removal immunostimulation action that resulted from irradiation.



Discussion 2

Radiation protection effects

- Significantly tumor growth inhibition was observed in *Enterococcus Faecalis 2001* administration group, both irradiation group and non-irradiation group.
- Significantly blood TNF- α , IFN- γ , increase of interleukin-12 were accepted to a *Enterococcus Faecalis 2001* administrated group.
- Increase of CD3/CD4, CD3/CD8, and CD3/CD16.
- It is thought that NK-cell is activated by *Enterococcus Faecalis 2001* administrated group.
- It is suggested that, because that is included in the *Enterococcus Faecalis 2001* of β -D-glucan 1-3,1-6 activates the T lymphocytes, it has enhanced the function of interferon.

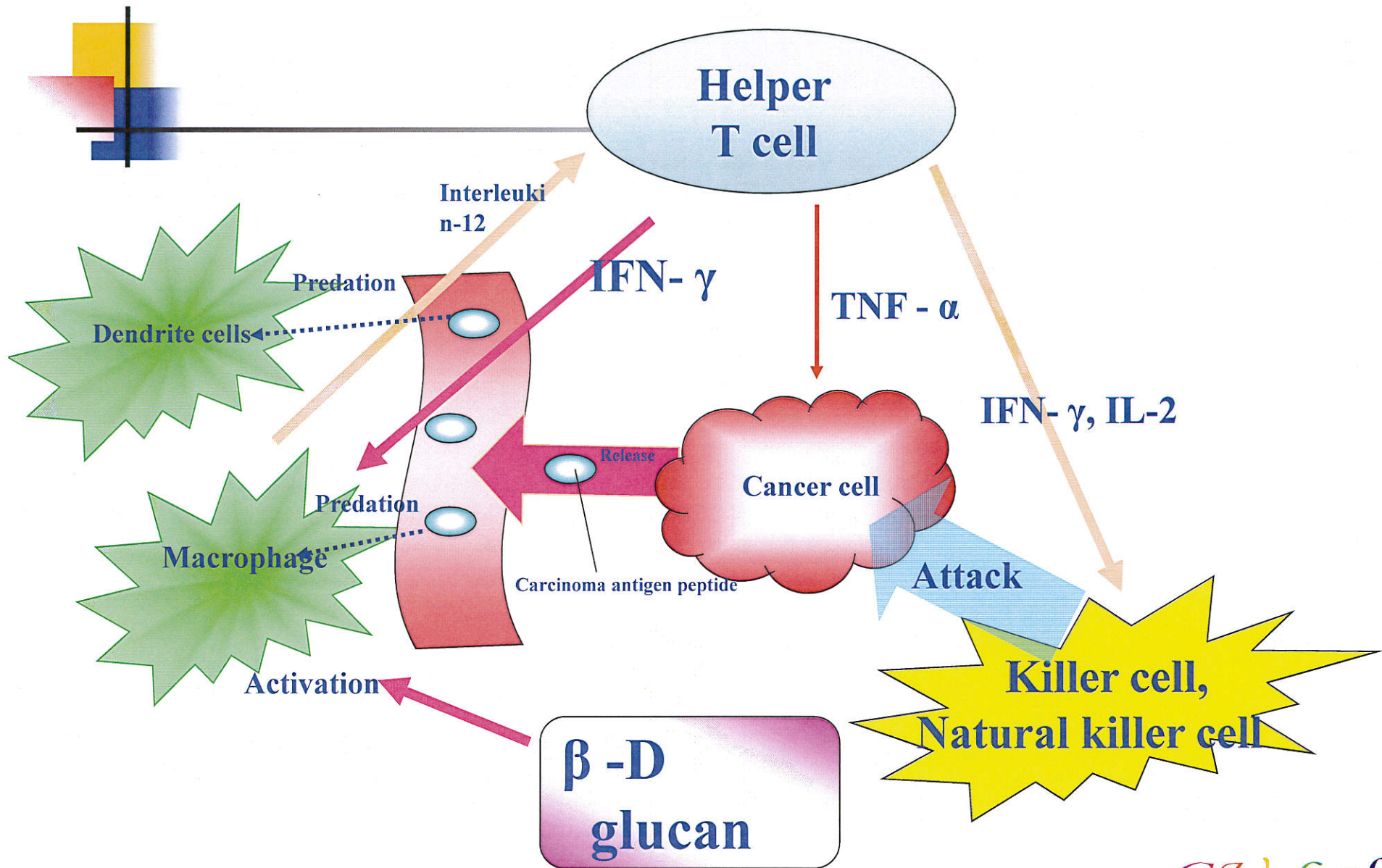


Discussion 3

Anti-tumor effect

- **1. Direct action**
- The reinforcement of TNF- α , on a tumor directly, and it is thought that antitumor effect is obtained.
- **2. Indirect action**
- The β -D-glucan which is an active principle of *Enterococcus Faecalis 2001* is absorbed than intestinal tract payer patches and it activate a macrophage and discharge interleukin-12.
- Helper T-cell is activated by this and lets IFN- γ increase and lets NK-cell and a macrophage activate more.
- It is thought that Th1 enhances these by a work of cytokine.
- On this account immunization enhancement, anti-inflammatory action act and deaden a cancer cell indirectly by raising self-cure power, and it is thought that antitumor effect was obtained.

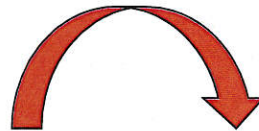
Mechanism of the anticancer effect by EF2001 of β -(1-3)-D glucan



Conclusions



- **Radiation protection effects**
- Immunostimulation action depends on activation such as helper T-cell or NK-cell than the meaningful reinforcement of IFN- γ , interleukin-12 of *Enterococcus Facalis 2001*.
- It is thought that is radical scavenger by the free radical removal that occurred by irradiation in *Enterococcus Facalis 2001* of antioxidant effects.
- **Antitumor**
- It was found that there were both sides of indirect action with activation such as activation of helper T-cell, NK-cell by a direct action, by the reinforcement of TNF- α and cytokine such as IFN- γ , interleukin-12.



***Enterococcus Facalis 2001* have anticancer effect and radiation protection effect.**