Effect of Extended Exposure of Low-dose Radiation on Autoimmune Diseases of Immunologically Depressed MRL/MpJ-gld/gld Mice

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ABSTRACT
We analyzed alterations of splenic T cell subpopulations and amelioration of autoimmune diseases of MRL/MpJ-gld/gld mice (MRL/gld mice) after the extended exposure to low-dose radiation (LDR). Four-month old MRL/gld mice were exposed to 0.05, 0.2 and 0.5 Gy/day for 4 weeks (5 days/week) with a total dose of 1, 4 and 10 Gy, respectively. The mice irradiated with 0.2 and 0.5 Gy/day showed an obvious decrease in the proportions of splenic CD4^+CD8^+ T cells and remission of their autoimmune diseases. In the mice irradiated with 0.2 Gy/day, apoptotic cells were found in the white pulp of the spleen after the last irradiation, but not in that of the treated MRL/MpJ-+/+ mice (MRL/wild type mice).

It seems that the accumulated CD4^-CD8^- T cells are more sensitive to radiation than other T cell subpopulations and prone to apoptosis, and efficient elimination of abnormal CD4^-CD8^- T cells by radiation-induced apoptosis may lead to the amelioration of autoimmune disease.

INTRODUCTION
James and Makinodan previously reported that whole-body exposure to 0.04 Gy/day of gamma-rays for 20 days changed the proportions of splenic T cell subpopulations to normal levels and improved the conditions of lymphadenopathy and splenomegaly in C57Bl/6J-lpr/lpr mice (C57Bl/lpr mice)(1). The lpr mice are known to have a mutation within the Fas gene (2, 3). Moreover, the autosomal recessive mutation induces autoimmune diseases, nephritis and arthritis, with lymphadenopathy and splenomegaly in approximately five or six-month old mice in the presence of MRL background (4-6). We used MRL/gld mice instead of C57Bl/lpr mice in this study. MRL/gld mice develop a similar phenotype of autoimmune disease as lpr mice with a similar mechanism at about four months of age but have the mutation within a different gene, FasL(7-9). Abnormal CD4^-CD8^- T cells that accumulated due to an insufficient Fas-FasL system induce these autoimmune diseases in lpr and gld mice.

In C57Bl/lpr mice, observed by James et al., it was thought that this phenomenon of radiation-induced apoptosis occurred specifically in the abnormal CD4^-CD8^- T cells, and their decrease then participated in the improvement of the conditions of autoimmune diseases.

MATERIALS AND METHODS
Animals
Female MRL/wild type mice as control for genetic background and MRL/gld mice (Japan SLC, Inc., Japan) were used. The mice were given laboratory feed (CE-2, Clea Japan, Japan) and water ad libitum and kept according to the Guiding Principles for the Care and Use of Animals approved in 1987 by the Faculty Meeting of the Univ. of Occup. and Environm. Health, Japan.

Irradiation
The mice were exposed to gamma-rays from Gammacell 40 Exactor (1.04 Gy/min.) (Nordion Intl. Inc., Canada) or 137Cs simulator (0.0013 Gy/min.)(Sangyoukagaku KK., Japan).

Four-month old MRL/gld mice were exposed to 0.05 Gy/day (dose rate; 0.0013 Gy/min.)(Group I), 0.2 Gy/day (dose rate; 1.04 Gy/min.)(Group II) and 0.5 Gy/day (dose rate; 1.04 Gy/min.)(Group III) for 4 weeks (5 days/week) with a total dose of 1 Gy, 4 Gy and 10 Gy, respectively. MRL/wild type mice, four months of age, were exposed to 0.2 Gy/day (dose rate;1.04 Gy/min.)(Group IV) and 0.5 Gy/day (dose rate; 1.04 Gy/min.)(Group V) for 4 weeks (5 days/week).

Pathological analysis of autoimmune disease
Unirradiated MRL/gld and MRL/wild type mice were sacrificed at four months of age and irradiated MRL/gld mice were sacrificed at four and five months of age. The organs indicated autoimmune diseases, i. e., salivary glands, kidneys and knee joints, were resected and analyzed pathologically. The salivary glands and kidneys were fixed in 10% formalin. The knee joints were treated with 10% formic acid-formalin solution or 14% EDTA (pH 7.4-7.6) solution for decalcification. Organ specimens were stained with hematoxylin and eosin.
Three-color Flow cytometry

The phenotypic expression of surface antigens on T cell subsets was determined by three-color flow cytometry. The mice irradiated to extended LDR were sacrificed at 3 days after last irradiation. An aliquot of spleen cell suspensions from irradiated and unirradiated mice was stained with Cy-chrome-conjugated rat anti-mouse monoclonal antibodies to CD3 (PHARMINGEN, USA), fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse monoclonal antibodies to CD4 (PHARMINGEN, USA) and R-phycoerythrin (R-PE)-conjugated rat anti-mouse monoclonal antibodies to CD8 (PHARMINGEN, USA). The treated cells were analyzed by EPICS-XL flow cytometer (Beckman Coulter, Inc., USA).

Apoptosis in spleen

The mice irradiated to extended LDR were sacrificed for analysis of apoptosis at 4 hours after last irradiation. The methods of detection for apoptosis were as described previously (10). Briefly, the spleens from treated and untreated mice were sectioned and stained with an Apop Tag DNA nick-end labeling kit (Oncor Inc., USA), which is a modified TUNL method (11).

RESULTS

All MRL/gld mice manifested lymphadenopathy and splenomegaly in macroscopy, and the proportions of splenic T cells subpopulations showed abnormality at four months of age (Fig. 1). In microscopy, arteritis of kidney and arthritis of knee joint were found (Data not shown). There was little incidence of glomerulonephritis and arteritis of the salivary gland.

In irradiated mice, Groups II & III (0.2 & 0.5 Gy/day) mice showed an obvious decrease in the proportion of CD4-CD8- splenic T cells and the worse decrease of CD4-CD8+ T cells was found in Group III (Fig. 1).

In MRL/wild type mice, there were a shift of the proportion of CD4+CD8- and CD4-CD8+ splenic T cells in Group V (0.5 Gy/day), but almost no effect on Group IV (0.2 Gy/day). A greatly decrease in the proportion of CD4+CD8+ T cells was found in Group III and Group V (Fig. 1). The reduction of the proportion of T cell subpopulations, observed in Groups II & III, was not found in Group I.

![Figure 1](image-url)

Figure 1. Proportion of splenic T cell subpopulations in irradiated and unirradiated groups. Each T cell subpopulation was detected by three-color flow cytometry as described materials and methods. Each bar indicates (■) CD4+CD8+, (□) CD4+CD8-, (▲) CD4-CD8-, (●) CD4-CD8+. The conditions of inflammation, such as arteritis of the kidney and arthritis of the knee joint observed in the control group, were ameliorated by extended exposure of low-dose radiation. In Group I, we were not able to find any effects such as the remission of autoimmune diseases and lymphadenopathy and splenomegaly. In
Group II, apoptotic cells were found in the white pulp of the spleen after last irradiation, but not in that of the irradiated and unirradiated MRL/wild type mice.

DISCUSSION

We meant to reconfirm the findings of James and Makinodan (1) using the same mice (C57BL/lpr) and elucidate the mechanisms of change in the proportions of splenic T cell subpopulations to normal levels after gamma-ray irradiation. Since our C57BL/lpr mice did not manifest severe symptoms similar to that described by James et al., we used MRL/gld mice which were regarded as indicating the most severe condition of autoimmune diseases with the same cause (12).

In this study, we found that the CD4-CD8- T cell subpopulation was the most sensitive to radiation (see Fig. 1, Groups II & III). In the white pulp of spleen, apoptotic cells were found in Group II after last irradiation but not found in Group IV. These results showed that abnormal CD4-CD8- T cells are of higher susceptibility to radiation-induced apoptosis. Judging from spleen weights (Table 1), the actual number of the CD4-CD8- T cells that scattered and accumulated on several organs seemed to reduce drastically. The reduction of number of the cells probably prevents lymphadenopathy on peripheral lymph nodes as we did not see swelling of peripheral lymph nodes of mice in Groups II and III. The reduction of CD4-CD8+ T cell subpopulations was also found in Group III and V (Fig. 1), this condition, 0.5 Gy/day for 4 weeks (5 days/week), might be considered an overdose.

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<th>Table 1. Spleen weight in irradiated and unirradiated mice (mg)</th>
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It was reported that arteritis of the kidney and arthritis of the knee joint in MRL/gld mice were curable using anti-FAS antigen (12). Abnormal CD4-CD8- T cells are Fas positive and apoptosis occurs in these cells due to the anti-FAS antigen. As a result of the investigation, the remission of autoimmune diseases was found. Although the cause of the reduction of abnormal CD4-CD8- T cells is different between their investigation and ours, the effects are similar regarding the reduction of CD4-CD8- T cells, which leads to the amelioration of autoimmune diseases.

Due to the extended exposure to low-dose radiation that we used in Groups II and IV, apoptosis occurs in CD4-CD8- T cells efficiently and has no effect on normal organs. It seems that the abnormal expansion CD4-CD8- T cells are more sensitive to radiation than the other subpopulations. The decreasing of the proportion of the CD4-CD8- T cells by radiation-induced apoptosis leads to the amelioration of autoimmune disease in MRL/gld mice.

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REFERENCES


