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Preventive and therapeutic effects of low-dose whole-body irradiation on collagen-induced rheumatoid arthritis in mice

Ji Young Kim[1,*], Yeong Ro Lee[1], Young Ae Lee[1], Chin-Hee Song[1], So Hyun Han[1], Seong Jun Cho[1] and Seon Young Nam[2]

[1]Radiation Effects Research Section, Radiation Health Institute, Korea Hydro & Nuclear Power Co., Ltd., Seoul 04505, Republic of Korea
[2]R&D Strategy & Planning Section, Radiation Health Institute, Korea Hydro & Nuclear Power Co., Ltd., Seoul 04505, Republic of Korea
*Corresponding author. Radiation Effects Research Section, Radiation Health Institute, Korea Hydro & Nuclear Power Co., Ltd., 38 Seosomun-ro Jung-gu, Seoul 04505, Republic of Korea. Tel: +82-2-61-06-44-10; Fax: +82-5-02-73-40-56-0; Email: elizabeth@khnp.co.kr
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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by progressive joint inflammation, resulting in cartilage destruction and bone erosion. It was reported that low-dose radiation modulates immune disease. Here, we investigated whether low-dose whole-body irradiation has preventive and therapeutic effects in collagen-induced RA (CIA) mouse models. Fractionated low-dose irradiation (0.05 Gy/fraction, total doses of 0.1, 0.5 or 0.8 Gy) was administered either concurrently with CIA induction by Type II collagen immunization (preventive) or after CIA development (therapeutic). The severity of CIA was monitored using two clinical parameters, paw swelling and redness. We also measured total Immunoglobulin G (IgG) and inflammatory cytokines (interleukine (IL)-6, IL-1β and tumor necrosis factor-alpha (TNF-α)) in the serum by enzyme-linked immunosorbent assay, and we evaluated histological changes in the ankle joints by immunohistochemistry and hematoxylin and eosin staining. Low-dose irradiation reduced CIA clinical scores by up to 41% in the preventive model and by 28% in the therapeutic model, while irradiation in the preventive model reduced the typical CIA incidence rate from 82 to 56%. In addition, low-dose irradiation in the preventive model decreased total IgG by up to 23% and decreased IL-1β and TNF-α by 69 and 67%, and in the therapeutic model, decreased total IgG by up to 35% and decreased IL-1β and IL-6 by 59 and 42% with statistical significance ($P < 0.01$, 0.05 and 0.001). Our findings demonstrate that low-dose radiation has preventive and therapeutic anti-inflammatory effects against CIA by controlling the immune response, suggesting that low-dose radiation may represent an alternative therapy for RA, a chronic degenerative immune disease.

Keywords: low-dose radiation; collagen-induced rheumatoid arthritis (CIA); pro-inflammatory cytokines; anti-collagen Type II IgG

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting 0.4–1.3% of adults worldwide, particularly women and older adults, and its incidence continues to increase [1]. RA is the most prevalent chronic inflammatory disease, characterized by persistent synovitis, systemic inflammation, progressive cartilage destruction and bone erosion [2]. RA pathophysiology remains mostly unexplained; however, many cytokines are active in the joints of RA patients. These cytokines play fundamental roles in the inflammation, articular destruction and comorbidities associated with RA [3]. RA treatments are divided into four main strategies: (i) nonpharmacological treatments, including surgery to remove or replace an affected joint or a combination of physical therapy and lifestyle changes; (ii) nonsteroidal anti-inflammatory drugs for reducing pain and stiffness; (iii) glucocorticoids, which offer rapid systemic disease-modifying effects but may be associated with long-term side effects and (iv) disease-modifying anti-rheumatic drugs targeting inflammation to prevent further joint damage and disease progression [1]. However, not all patients benefit from these

treatments, some of which are associated with severe complications (e.g. spine and lung disease [4–7]) and adverse effects (e.g. cardiovascular, gastrointestinal ulceration, nausea and osteoporosis) [8]. The progression of RA can cause patients to suffer from considerable side effects, high financial costs and a decreased quality of life. Therefore, the development of new treatments that address these shortcomings remains necessary.

In contrast to high-dose radiation, low-dose ionizing radiation has shown many beneficial effects in various disease-related models; however, the underlying immunological and molecular mechanisms have not been fully explored [9–14]. For decades, the use of low-dose radiation therapy has been clinically documented as exerting effects on benign, inflammatory and chronic degenerative diseases. In particular, radiation therapy for osteoarthritis (OA) is commonly used in Germany, and evidence-based practice guideline for local irradiation has also been established nationwide. In addition, ∼65–75% of patients after radiotherapy were reported to have shown good and excellent therapeutic effects [15]. Meanwhile, there are few clinical case reports of radiation therapy for RA, a systemic inflammatory disease, but recently, results of the RA patients have been reported to be cured by radiation therapy in Japan [16]. As such low-dose radiation therapy has gained attention in the field of radiotherapy. Low-dose radiation therapy administered at a single dose of 0.5 Gy has no harmful effects on key cell types in healthy joints [17]. In the current study, we would like to find out whether 0.05 Gy radiation, which is lower than 0.5 Gy, is effective in treating and preventing RA symptoms even when irradiated twice, 10 times and 16 times using collagen-induced RA (CIA) mouse model.

MATERIALS AND METHODS

Generation of collagen-induced RA mouse models

Male DBA/1J mice (8 weeks of age) were purchased from the Shizuoka Laboratory Animal Cooperation (Shizuoka, Japan) and maintained in pathogen-free conditions. All procedures were approved by the Institutional Animal Care and use Committee of the Radiation Health Institute of Korea Hydro and Nuclear Power Company. Mice were treated in accordance with governmental guidelines and the guidelines of the Radiation Health Institute for the care of animals. Immunization-grade bovine Type II collagen (CII; Chondrex, USA) was dissolved in 0.01 N acetic acid to a concentration of 2.5 mg/ml at 4°C overnight with tumbling rotation. Aliquots were stored at −70°C. Collagen aliquots were thawed slowly on ice. An emulsion was prepared by combining equal volumes of CII and complete Freund's adjuvant (Chondrex, USA) and stored on ice until use. Mice were anesthetized and injected intradermally at the base of the tail with 100 μl emulsion containing 125 μg CII. On Day 21 after the primary immunization, mice received a booster shot in the footpad consisting of 100 μg CII emulsified in an equal volume of incomplete Freund's adjuvant (Chondrex, USA). RA developed by approximately Day 49. Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group.

Low-dose radiation therapy for collagen-induced RA mouse model

CIA mice were administered whole-body irradiation (0.05 Gy/fraction) using a ^{137}Cs γ-ray irradiator (Gammacell® 40 Exactor, Best Theratronics, Ltd, Canada) at a rate of 0.6 Gy/min. Radiation was administered three times per week to deliver total radiation doses of 0.5 and 0.8 Gy. However, the total radiation dose of 0.1 Gy was irradiated in only the first two times. The schedules for immunization and irradiation are shown in Fig. 1. A preventive CIA model, in which primary immunization and irradiation both commenced on Day 0 (Fig. 1A), was generated to determine whether radiation could prevent or delay RA symptom development. A therapeutic CIA model, in which radiation therapy began after RA symptoms developed (∼49 days post-primary immunization; Fig. 1B), was generated to determine whether radiation could suppress RA symptoms.

Clinical assessment

Starting on Day 21 after primary immunization (immediately after secondary immunization), mice were monitored three times per week for CIA severity using two clinical parameters: paw swelling and redness. Paw thickness was measured using a digital caliper (Mitutoyo Corporation, Japan). Severity was evaluated using an established scoring system: 0 = normal; 1 = mild but definite redness and swelling of the ankle or wrist or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; 2 = moderate redness and swelling of the ankle or wrist; 3 = severe redness and swelling of the entire paw, including the digits; and 4 = maximally inflamed limb with involvement of multiple joints [18]. Blood was collected from CIA mice following euthanasia, and serum was separated and stored at −80°C until analysis.

Incidence of RA

The incidence of RA was measured only in the preventive model. The clinical symptoms caused by CIA were confirmed every day from the 35 days since the start of the experiment. The incidence of RA was expressed by calculating the number of mice showing symptoms as a percentage from the total number of mice used in the experiment by date.

Collagen Type II-specific IgG antibody assay

Anti-CII antibody levels in CIA mice sera were measured using the Mouse IgG ELISA Quantification Set (Bethyl Laboratories, Germany) and ELISA Starter Accessory Kit (Bethyl Laboratories, Germany). Briefly, a 96-well plate was incubated overnight at 4°C with bovine CII (4 μg/ml) in coating buffer. The plate was washed five times with washing solution and then incubated with blocking buffer for 30 min at room temperature. After washing, the plate was incubated with serum samples (1:1000 dilution for negative control group, 1:10000 dilution for treatment group) or serially diluted standard for 60 min at room temperature. Following washing, the plate was incubated with horseradish peroxidase-conjugated goat anti-mouse IgG detection antibody for 60 min at room temperature. After a final wash, the plate was developed with 3,3′,5,5′-tetramethylbenzidine in the dark for 15 min. The reaction was stopped with stop solution. The absorbance was measured at 450 nm with a spectrophotometer (Labsystems, Helsinki, Finland).

Determination of cytokine levels

Blood from each mouse on the final day of the experimental period was collected and centrifuged at 3000 rpm for 25 min, and sera was

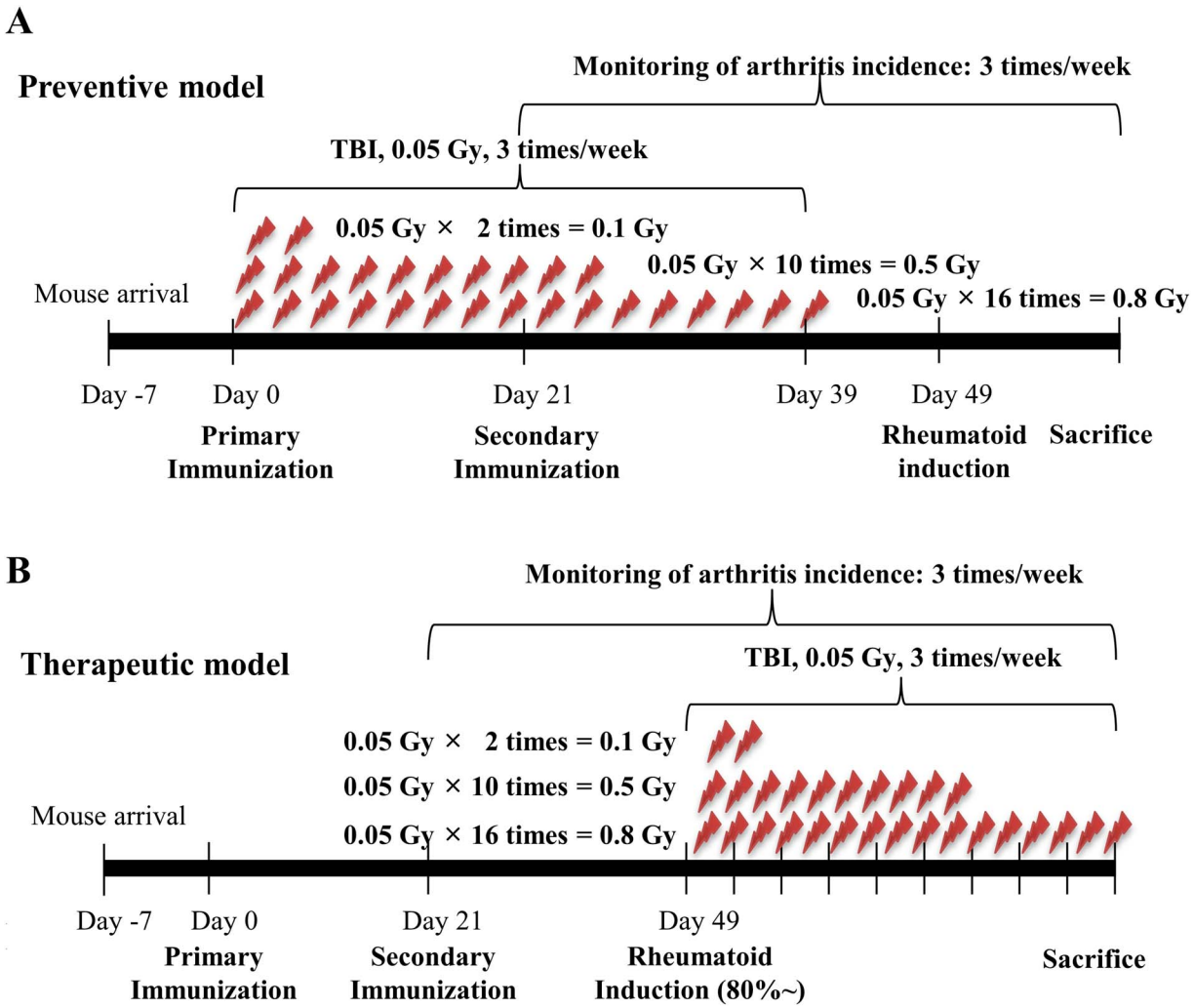

Fig. 1. Immunization and irradiation schedules. DBA/1J mice were immunized with CII in complete Freund's adjuvant to generate CIA mouse models. After 3 weeks, a second immunization with CII in incomplete Freund's adjuvant was administered. For the preventive CIA model (A), irradiation and primary immunization both commenced on Day 0. For the therapeutic CIA model (B), irradiation was performed after RA symptoms were established. Whole-body irradiation (0.05 Gy/fraction) was administered three times per week using a ^{137}Cs γ-ray irradiator (Gammacell® 40 Exactor) to deliver total radiation doses of 0.5 and 0.8 Gy. However, the total radiation dose of 0.1 Gy was administered only the first two times. Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group.

kept at −80°C until use. The concentrations of interleukin (IL)-6, IL-1β and tumor necrosis factor-alpha (TNF-α) in the sera isolated by above method were measured using the Quantikine ELISA kit (R&D Systems, USA), according to the manufacturer's protocol. Standard curves were generated through serial dilution of the recombinant IL-6, IL-1β and TNF-α. The detectable ranges of IL-6, IL-1β and TNF-α were 7.8–500 pg/ml, 12.5–800 pg/ml and 10–700 pg/ml, respectively.

Histological analysis

Following euthanasia, mouse ankles were removed, fixed in 4% paraformaldehyde (Sigma, USA) and decalcified with Calci-Clear Rapid Solution (National Diagnostics, USA). The ankles were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) (Sigma, USA) for histological examination.

For immunohistochemistry, ankle specimens were blocked with 3% methanolic hydrogen peroxide and then digested. Formalin-fixed, paraffin-embedded tissues were exposed to pepsin (Invitrogen, USA; 1:200) by dropping it on the slide at 37°C for 10 min to unmask antigens. After blocking with 3% bovine serum albumin, the sections were incubated with primary rat antibodies against cartilage oligomeric matrix protein (COMP; 1:50, Antibodies Online, Germany), cyclooxygenase-2 (COX2; 1:100, Santa Cruz Biotechnology, USA), IL-6 (1:50, Antibodies Online, Germany), matrix metalloproteinase-3 (MMP3; 1:100, Santa Cruz Biotechnology, USA), MMP14 (1:100, Abcam, England) and TNF-α (1:100,

Santa Cruz Biotechnology, USA). Staining was visualized using immunoperoxidase (BD Biosciences, USA).

Statistical analysis

Data are presented as the mean ± standard error of the mean. Single comparisons between two experimental groups were performed using the unpaired Student's *t*-test. Differences were deemed significant when the *P*-value was <0.05.

RESULTS

Low-dose radiation attenuates clinical symptoms of collagen-induced RA in mice

We generated CIA mouse models to investigate the preventive and therapeutic effects of low-dose radiation on RA symptoms (Fig. 1). The severity of RA was measured subjectively using a scoring system. The first weak signs of RA appeared after the second immunization (Day 21), and the incidence of CIA was ∼80% by Day 49. To investigate the preventive effects of low-dose radiation, CII immunization and irradiation both commenced on Day 0. Preventive low-dose radiation reduced the RA clinical scores assessed for the back paws (Fig. 2A) and all paws (Fig. 2B) in the CIA model mice. The front paw was small in size, so there was no significant change in the clinical score, and on average, it measured <1. Therefore, only clinical score results for the back and all paws were reported. The clinical score in the back paw of CIA mice decreased from 2.2 ± 0.25 to 1.6 ± 0.38, 1.7 ± 0.33 and 1.4 ± 0.30 by irradiation (Fig. 2A), and in the all paws, the clinical score decreased from 3.0 ± 0.34 to 1.9 ± 0.45, 1.9 ± 0.40 and 1.7 ± 0.41. In particular, it was reduced by up to 41% with statistical significance in the 0.8 Gy irradiation group (Fig. 2B). Figure 2C showed the back paw photographs taken at different directions. In addition to, irradiation also reduced RA incidence (Fig. 3). RA incidence indicates the proportion of mice with RA symptoms among all mice used in the experiment regardless of the severity of the symptoms. Although RA incidence increased to 82% on 49 days after the start of experiment, preventive radiation therapy reduced this effect (0.1 Gy, 66%; 0.5 Gy, 68%; 0.8 Gy, 59%). In particular, preventive radiation delivered at a dose of 0.5 Gy significantly reduced RA incidence from 34 to 13% through approximately Day 41. After 41 days, the RA incidence decreased for all radiation doses compared with the untreated CIA control (Fig. 3).

We also examined whether low-dose radiation therapy could alleviate the severity of established RA symptoms in the therapeutic CIA mouse model. Most CIA mice presented with RA symptoms, such as redness and swelling of the ankle or wrist and deformity of the joint, by 49 days after the primary immunization. After the onset of RA symptoms on the 49 days, low-dose irradiation reduced the redness and swelling of the paws and ankles but did not cure the joint deformities (Fig. 4). In particular, at doses 0.1 and 0.5 Gy, therapeutic radiation reduced RA clinical scores with statistical significance depending on the dose, while at 0.8 Gy, the clinical score increased independently of the radiation dose.

Low-dose radiation reduces anti-collagen Type II IgG levels in mouse serum

In the preventive model, the increased anti-CII IgG antibody showed a slightly reduced pattern by irradiation and was significantly reduced by 0.8 Gy irradiation (Table 1). By contrast, the anti-CII IgG levels were the lowest for therapeutic CIA mice that received a radiation dose of 0.1 Gy (0.05 Gy/fraction × 2), whereas no reduction in anti-CII antibody levels was observed in therapeutic CIA mice receiving a radiation dose of 0.8 Gy (0.05 Gy/fraction × 16).

Low-dose radiation reduces inflammatory cytokines level

To determine whether low-dose radiation affects the inflammatory process by regulating cytokine secretion, we measured serum levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in preventive and therapeutic CIA mice. Pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α are expressed in the arthritic joints in both CIA mouse model and human RA, and blockade of these molecules results in amelioration of disease. The levels of all three cytokines significantly increased in CIA mice. In the preventive CIA mice model, IL-1β and TNF-α levels were suppressed with statistical significance by multiple low-dose radiation (0.1, 0.5 and 0.8 Gy), and IL-6 was found to have no statistical significance and only a reduction effect (Table 1). In the therapeutic CIA mouse model, IL-1β was reduced with statistical significance by all irradiation (0.1, 0.5 and 0.8 Gy), but IL-6 was suppressed at 0.1 Gy and increased slightly at 0.5 and 0.8Gy. In the case of TNF-α, only a slight reduction effect was observed at all doses (Table 1).

Low-dose radiation induces histological changes caused by collagen-induced arthritis mice

We next examined histological changes in the ankle joints of preventive (Fig. 5) and therapeutic (Fig. 6) CIA mouse models. As shown in Figs 5G and 6G, inflammation and tissue destruction were not detected in the ankle joints of control mice; however, the ankle joints of CIA mice were severely eroded, and the spaces narrowed. These histological changes were inhibited by low-dose radiation in both treatment models. The cause of RA is uncertain, but changes of components in the joint in the event of an outbreak changes are well known. In particular, the increase in inflammatory cytokines IL-6 and TNF-a, inflammatory enzyme COX-2, protease MMPs and COMP is known to cause severe joint damage, so we have confirmed it through immunohistochemistry staining, and their expression was shown in brown. Immunohistochemical analysis of joints from untreated CIA mice revealed markedly increased expression of inflammatory cytokines, inflammatory enzymes, protease related to joint inflammation and cartilage and bone destruction, such as COMP (Figs 5A and 6A), COX2 (Figs 5B and 6B), IL-6 (Figs 5C and 6C), MMP3 (Figs 5D and 6D), MMP13 (Figs 5E and 6E) and TNF-α (Figs 5F and 6F). Moreover, inflammatory factors expression levels increased in untreated CIA mice but were reduced in both radiation-treated CIA mouse models.

DISCUSSION

Unlike high-dose radiation, low-dose radiation has beneficial health effects, including anti-inflammatory, anti-oxidative and immune-enhancing effects, attributed to the stimulation of biologically protective mechanisms. We previously showed that low-dose radiation

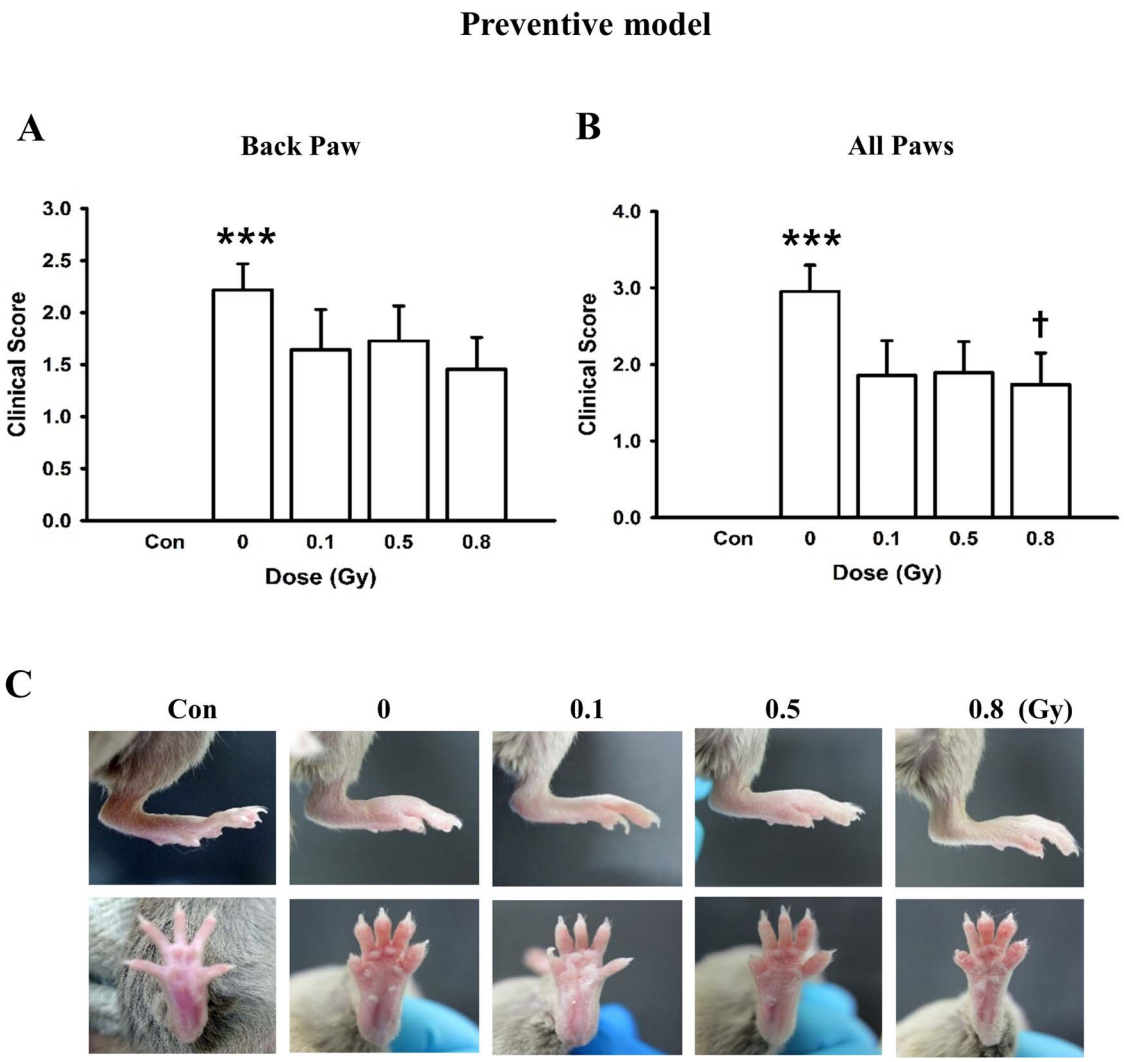

Fig. 2. The preventive effects of low-dose radiation on the clinical score. After the second immunization, mice were monitored three times per week for the severity of CIA using two parameters (paw swelling and clinical score). Severity was evaluated using the following scoring system: 0 = normal; 1 = mild but definite redness and swelling of the ankle or wrist or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; 2 = moderate redness and swelling of ankle and wrist; 3 = severe redness and swelling of the entire paw, including the digits; and 4 = maximally inflamed limb with involvement of multiple joints. Photographs of paw were taken in the day of euthanasia. Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group. Data represent means ± SEM from three independent experiment. (A) Clinical score for the back paw. (B) Clinical score for all paws. (C) Photographs of back paws from different directions. Con = control. Con vs 0 Gy: *** = $P < 0.001$; 0 Gy vs 0.8 Gy: † = $P < 0.05$.

therapy inhibits allergic reactions by regulating the hypersensitivity immune response in cell and mouse models of antigen–antibody reactions [9, 12, 14, 19].

RA is an autoimmune disease in which cartilage or bone is destroyed and deformed due to inflammation of the synovial membrane of joint tissue due to abnormal immune responses, and OA is a disease caused by physical damage to cartilage due to aging and excessive use. In the end, both diseases have symptoms such as pain caused by inflammation, but the cause of the disease is different. Therefore, in this study, to observe the therapeutic and preventive effects on RA, an autoimmune disease, the experiment was conducted using whole-body radiation instead of local radiation used to treat OA. Because no cure exists for RA, a chronic, systemic, degenerative immune disease characterized by the persistent inflammation of synovial membranes, we investigated whether low-dose radiation could exert preventive and therapeutic effects by controlling the immune response in the CIA mouse model, similar to our observations in the allergic reaction model, an autoimmune disease.

Table 1. The effects of low-dose radiation on total IgG and pro-inflammatory cytokines in the preventive and therapeutic CIA mouse model

Group (Gy)	Preventive model				Therapeutic model			
	Total IgG[a] (ng/ml)	IL-1ß[b]	IL-6[b] (pg/ml)	TNF-α[b]	Total IgG[a] (ng/ml)	IL-1ß[b]	IL-6[b] (pg/ml)	TNF-α[b]
Con	71.2 ± 0.70	16.1 ± 1.05	5.3 ± 4.79	1.9 ± 0.33	76.3 ± 7.05	8.5 ± 1.16	4.6 ± 1.46	4.6 ± 1.14
0	137.6 ± 8.15***	44.8 ± 4.64***	129.3 ± 33.43**	48.2 ± 5.21***	257.2 ± 21.59***	37.0 ± 5.09***	121.0 ± 16.41***	31.6 ± 5.24***
0.1	127.9 ± 5.39	22.3 ± 2.03††	62.2 ± 15.54	19.6 ± 1.14††	165.9 ± 12.01††	21.1 ± 1.30†	69.6 ± 10.10†	21.8 ± 1.66
0.5	122.6 ± 4.52	23.9 ± 4.70††	55.8 ± 12.50	16.1 ± 0.99†††	191.7 ± 14.61††	15.2 ± 2.14††	79.8 ± 19.58	19.1 ± 3.45
0.8	107.1 ± 6.29††	14.1 ± 1.95†††	70.0 ± 16.68	18.7 ± 0.75†††	233.1 ± 20.54	17.9 ± 3.42†	92.4 ± 20.69	21.0 ± 2.43

Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group. Data represent means ± SEM from three independent experiment. Con = control. Con vs 0 Gy: ** = $P < 0.01$, *** = $P < 0.001$; 0 Gy vs 0.1 or 0.5 or 0.8 Gy: $\dagger\dagger$ = $P < 0.01$, $\dagger\dagger\dagger$ = $P < 0.001$.
[a]Anti-CII IgG antibody levels in the serum of preventive and therapeutic CIA mouse models were measured by enzyme-linked immunosorbent assay. [b]The levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in serum from CIA model by enzyme-linked immunosorbent assay.

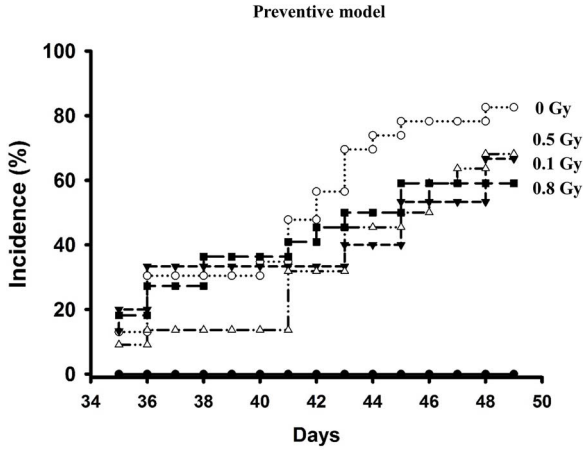

Fig. 3. The preventive effects of low-dose radiation on RA incidence. After the second immunization, the incidence of RA was measured. At least 15 mice were included in each group. Con = control.

The main protein in the joint cartilage is CII, and the immune response produced by CII is mainly targeted at joints. The production of CII-specific antibodies in mice is an important feature reported in RA, and the CIA mouse model is most commonly used because it can be made relatively similar to the pathogenicity of human RA and quickly [20]. The treatment dose and schedule of the radiation used in the experiment were selected according to the following criteria. In the previous mast cell model studies, 0.05 Gy, a dose that can be irradiated within a minimum error range and effective in controlling immune response, was selected as the fracted irradiation dose, and the treatment schedule was conducted three times a week, the same as the current schedule used in OA patients in Germany, and the final dose was 0.1, 0.5 and 0.8 Gy. Unlike of OA, RA is a systemic inflammatory disease, so radiation was irradiated throughout the body as in clinical trials in Japan [16].

The preventive model assigned mice to the group even before the start of the experiment, conducted irradiation and immunization and observed symptoms on the 49th day after the start of experiment. In the case of the therapeutic model, about 80% of mice developed RA on Day 49, so only mice with similar clinical score symptoms, except for mice that didn't express RA symptoms, were randomly assigned to the group on Day 49, and the effect was observed after irradiation. The experimental results used the average of each mouse. The cytokines and IgG level of mice that did not cause RA symptoms were significantly lower than those of mice that were induced. Both preventive and therapeutic models observed the effect until 1 month after the irradiation was completed, confirming that the effects were maintained, and at that time, the mice were sacrificed and obtained samples and conducted experiments.

Nakatsukasa *et al.* reported that DBA/1J mice with CIA treated with low-dose gamma radiation (0.5 Gy per week for 5 weeks) showed significant improvements in clinical RA symptoms, concomitant with reductions in serum anti-CII antibody and inflammatory cytokine (TNF-α, IFN-γ and IL-6) levels [21]. Because low-dose radiation is usually defined as <0.1 Gy, we investigated the preventive and therapeutic effects of low-dose irradiation delivered as fractionated doses of 0.05 Gy three times per week to achieve total radiation doses of 0.1, 0.5 and 0.8 Gy. We found that preventive low-dose radiation reduced the incidence rate of RA, and therapeutic low-dose radiation alleviated the clinical symptoms of established RA. Thus, very low-dose radiation could prevent and treat RA, similar to other inflammatory diseases.

Autoantibodies, such as rheumatoid factors and anti-CII antibodies, activate the complement cascade and recruit inflammatory cells into joints. Inflammatory cells produce key cytokines such as TNF-α, IL-1β and IL-6 involved in RA that drive inflammation, causing joint damage. TNF-α triggers the production of other cytokines and stimulates collagenase, stromelysin and osteoclast differentiation [22–24]. Cytokine regulation is of crucial importance in RA. Although TNF-α alone is not very destructive, it can synergistically enhance the destructive behaviors of IL-1β and IL-6 during chronic joint inflammation and concomitant erosive changes in cartilage and bone [25–27]. In our current study, low-dose radiation reduced serum levels of anti-CII antibody and inflammatory cytokines (TNF-α, IL-1β and IL-6) in CIA model mice (Table 1). In particular, low-dose radiation significantly reduced secreted IL-1β levels in both the preventive and therapeutic CIA models. In the preventive and therapeutic RA

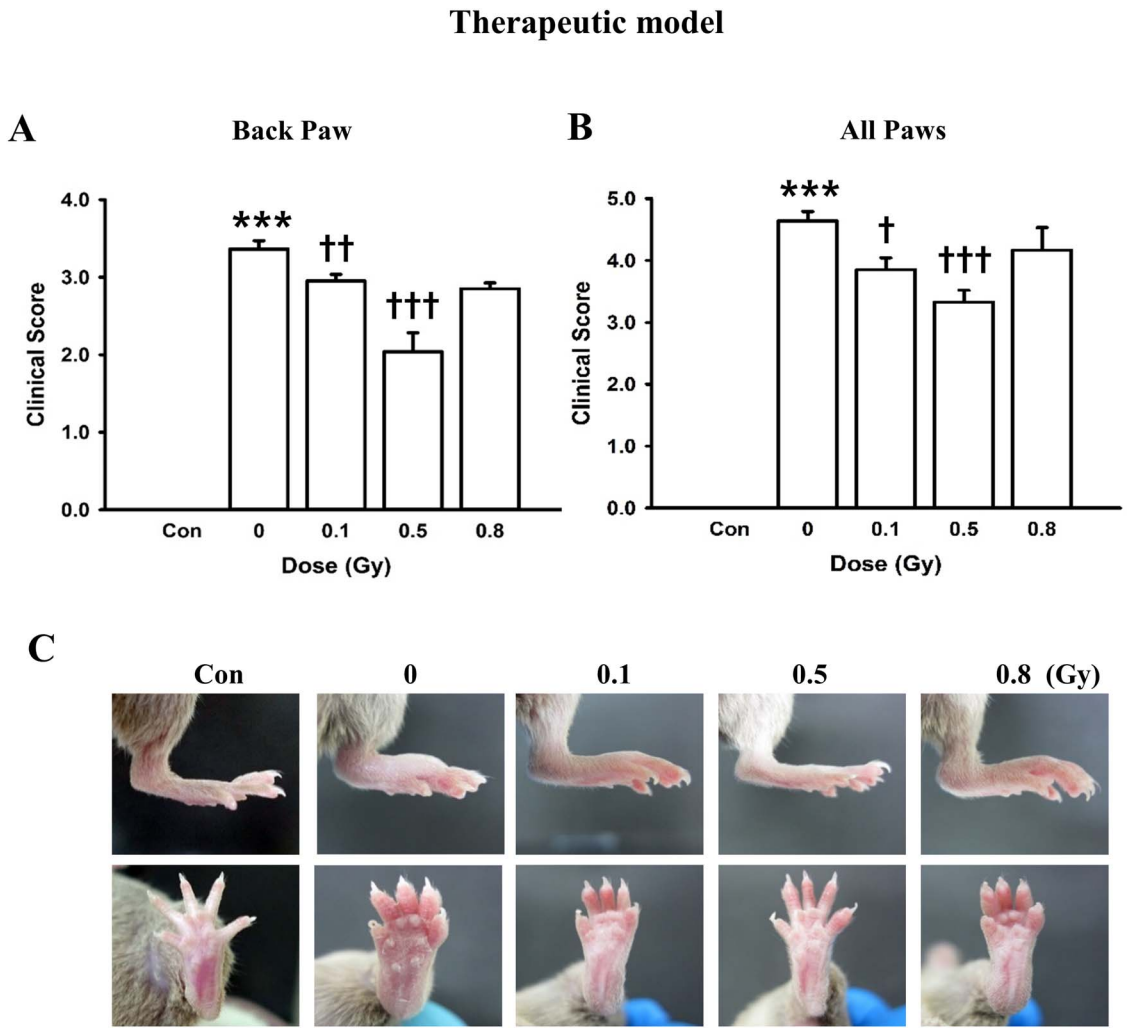

Fig. 4. The therapeutic effects of low-dose radiation on the clinical score. After generating the CIA model, radiation therapy was administered, and the severity of CIA was monitored three times per week using two parameters (paw swelling and clinical score). Severity was evaluated using the following scoring system: 0 = normal; 1 = mild but definite redness and swelling of the ankle or wrist or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; 2 = moderate redness and swelling of ankle and wrist; 3 = severe redness and swelling of the entire paw, including the digits; and 4 = maximally inflamed limb with involvement of multiple joints. Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group. Data represent means ± SEM from three independent experiment. (A) Clinical score for the back paw. (B) Clinical score for all paws. (C) Photographs of back paws from different directions. Con, control. Con vs 0 Gy: *** = $P < 0.001$; 0 Gy vs 0.1 or 0.5 Gy: † = $P < 0.05$, †† = $P < 0.01$, ††† = $P < 0.001$.

model, increased IL-6 cytokine in sera was reduced by irradiation. In particular, in the therapeutic model, it decreased with statistical significance at 0.1 Gy and increased slightly at 0.5 and 0.8 Gy but was not significant due to changes within the standard error range. When IL-6, anti-CII antibodies and clinical score were comprehensively interpreted, unlike the preventive effect, it was confirmed that there was no therapeutic effect at 0.8 Gy compared to 0.1 and 0.5 Gy. Therefore, it is considered very important to select the radiation dose when treating RA.

Thus, low-dose radiation can ameliorate arthritic symptoms by inhibiting anti-CII antibodies and pro-inflammatory cytokines, which are important players in RA development. These anti-inflammatory and anti-rheumatic effects suggest that low-dose radiation therapy may be an effective and safe alternative to pharmacological therapies for different inflammatory conditions.

Our previous study [9] showed that low-dose radiation is an effective immunomodulator in preventive and therapeutic allergy models. In Germany, low-dose radiation is commonly used to treat noncancer disease, especially degenerative arthritis [15], and a clinical trial is evaluating the effect of low-dose radiation in RA patients [16]. Therefore, we suggest that very low-dose radiation should be prioritized as an alternative treatment for degenerative disorders, such as RA, with enormous economic, social and ethical implications.

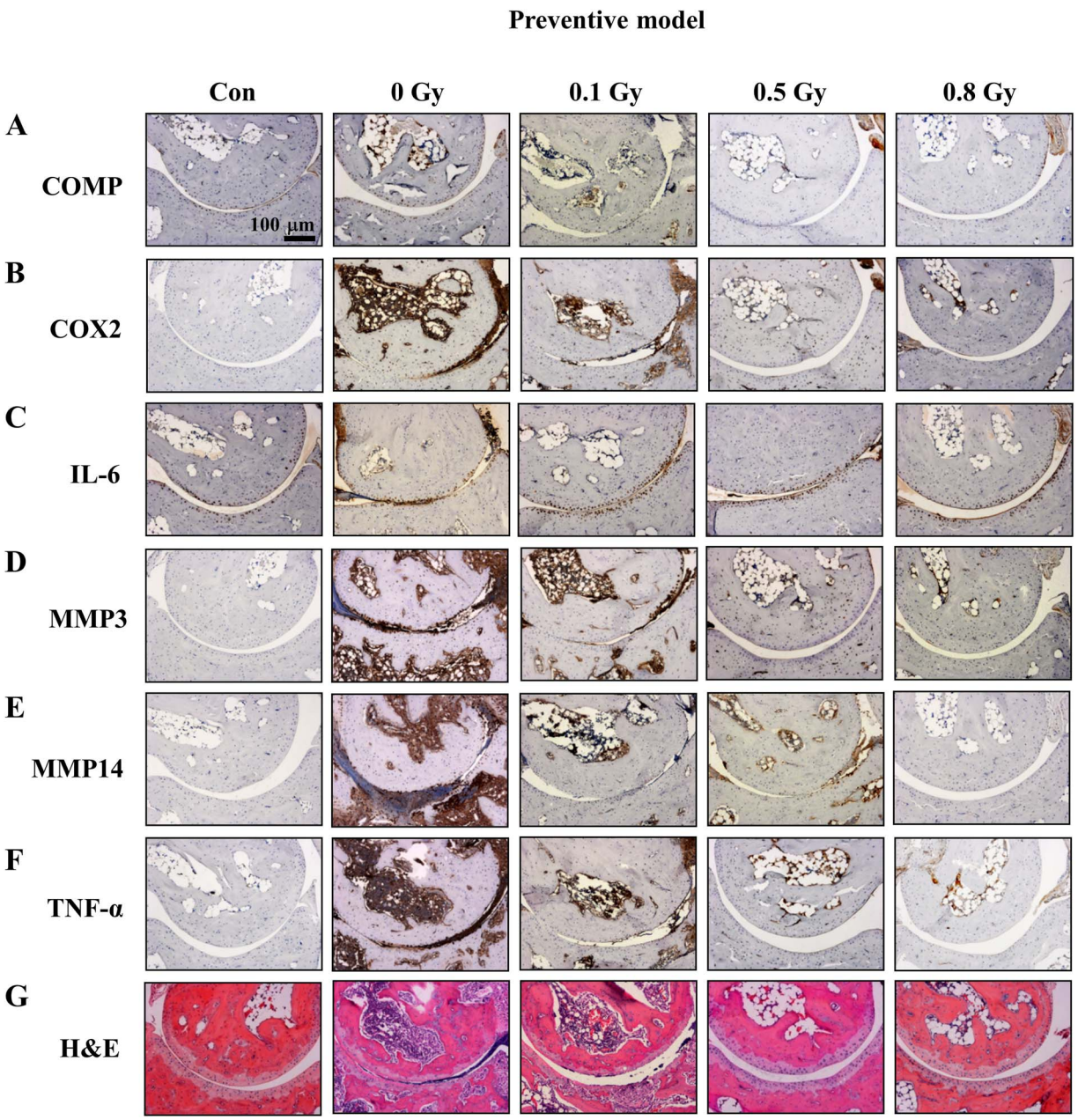

Fig. 5. Effect of preventive low-dose radiation on the histological changes in CIA mouse models. (A–G). Representative images are presented for the histological and immunohistochemical analysis of knee joint sections from CIA mice with or without preventive low-dose radiation therapy. A control group (Con) was not immunized with CII or treated with radiation. The severity of arthritis in the preventive CIA mouse model was assessed by staining for (A) COMP, (B) COX-2, (C) IL-6, (D) MMP3, (E) MMP14, (F) TNF-α and (G) H&E. Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group.

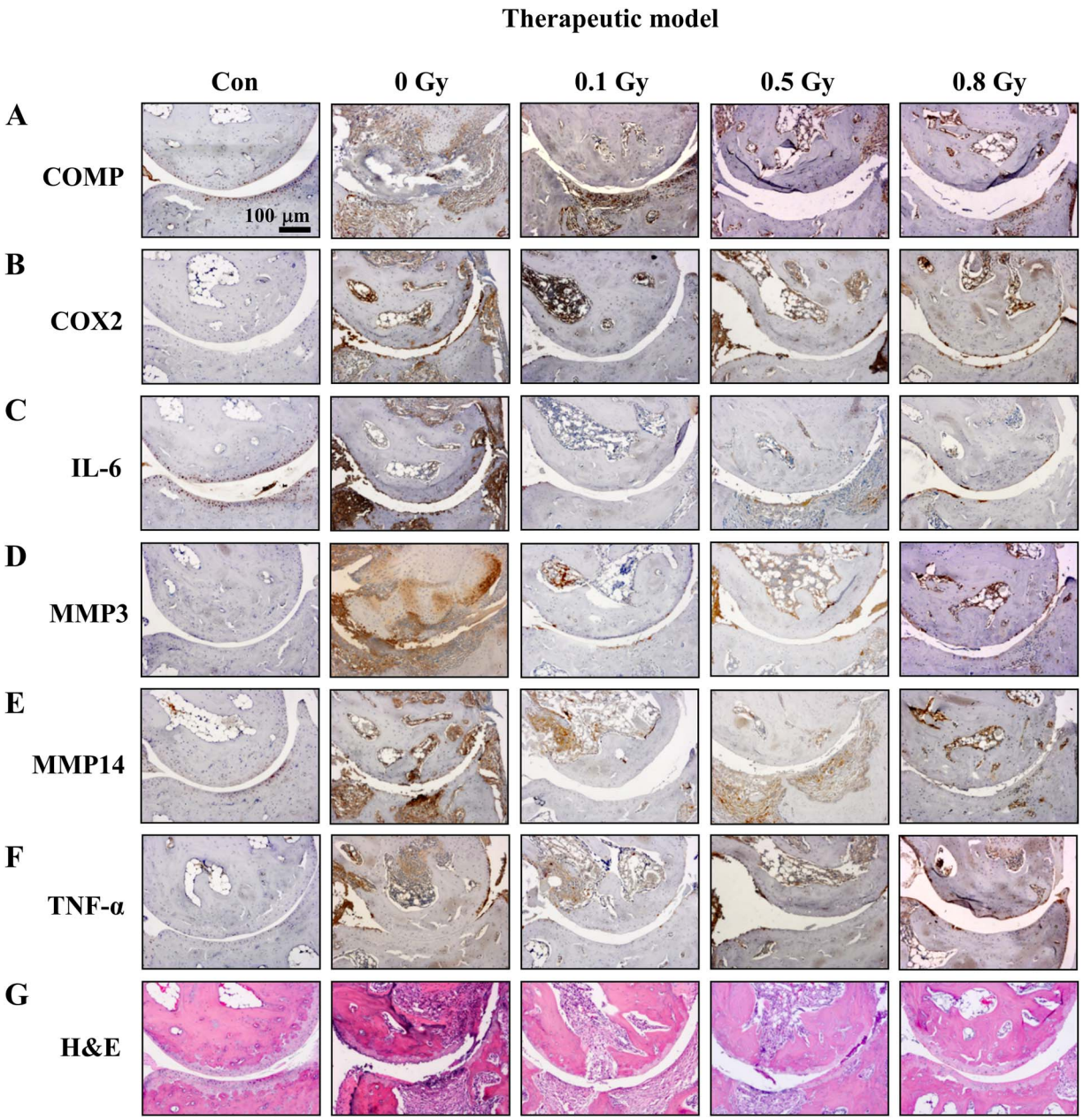

Fig. 6. Effect of therapeutic low-dose radiation on the histological changes in CIA mouse models. (A–G). Representative images are presented for the histological and immunohistochemical analysis of knee joint sections from CIA mice with or without therapeutic low-dose radiation therapy. A control group (Con) was not immunized with CII or treated with radiation. The severity of arthritis in the therapeutic CIA mouse model was assessed by staining for (A) COMP, (B) COX-2, (C) IL-6, (D) MMP3, (E) MMP14, (F) TNF-α and (G) H&E. Four mice were assigned to each group and replicated three or more times. Therefore, at least 12 mice were used per group.

CONFLICT OF INTEREST

There are no conflicts of interest.

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