Effects of irradiation on cytokine production in a mouse model of Behçet’s disease

S. Kang1, E.-S. Lee2, B. Choi3, H.C. Lim3, M. Chun1, S. Lee2, S. Sohn3,4

1Department of Radiation Oncology, 2Department of Dermatology, Ajou University School of Medicine, Suwon; 3Laboratory of Cell Biology, Ajou University Institute for Medical Sciences, Suwon; 4Brain Korea 21 Project for Medical Science, Suwon, South Korea.

Abstract

Objective
Low-dose whole-body irradiation is known to have anti-inflammatory effects. The objectives of this study were to verify that cytokine augmentation is induced by irradiation in vivo, and to assess the effectiveness of radiation in treating Behçet’s disease (BD).

Methods
Whole-body and half-body irradiation with single doses of 10cGy, 2Gy and 10Gy were delivered to normal mice, and cytokine and chemokine levels were analyzed in PBMC and sera. BD-like mice were treated with low-dose, half-body 10cGy irradiation.

Results
In normal mice, PBMC cytokine mRNA levels peaked four days after irradiation. Of the cytokines and chemokines examined, the levels of IL-4, IL-6, IL-12p40, TNF-α, TGF-β, MIP-1α and IL-18 were all influenced by radiation treatment. Of these, IL-4, an ameliorating factor for BD, was the most elevated following low-dose irradiation (10cGy group). FACS analysis showed intracellular IL-4-staining of 7.24±0.92% of PBMC from irradiated mice compared to 1.3±0.1% from non-irradiated, normal mice (p<0.005). Serum IL-4 levels were also significantly increased (6.08±1.7 pg/ml) relative to control (1.83±0.8; p<0.005).

Conclusion
Augmentation of cytokine production may contribute to the anti-inflammatory effects of low dose irradiation and amelioration of BD symptoms in this mouse model.

Key words
Low-dose irradiation, cytokine, mouse model, Behçet’s disease, anti-inflammation.
Introduction
Low-dose, whole body irradiation (1-3 Gy) is currently used to reduce non-relapse mortality in the treatment of patients with various hematological malignancies who are not considered as candidates for conventional hematopoietic cell transplantation (such as elderly patients, patients with pre-existing comorbidities or heavily-treated relapsed patients) (1-4). Radiation-induced immune enhancement is one of the mechanisms suggested for the success of this treatment (5, 6). However, the precise biological effects of low-dose irradiation are complex and poorly understood. Low-dose radiotherapy selectively reduces peripheral blood mononuclear cell (PBMC) adhesion to the endothelium in vitro, and induces PBMC apoptosis in a discontinuous, dose-dependent manner (7). This apoptosis may contribute to the anti-inflammatory effects of low-dose radiotherapy. Furthermore, low-dose radiotherapy (0.3-0.7Gy) induces maximal transforming growth factor (TGF) β production by endothelial cells, and thus, reduces endothelial cell stimulation. The down-regulation of leukocyte/PBMC adhesion to the endothelium may also contribute to the anti-inflammatory effects of radiation (8).

Behçet’s disease (BD) is a chronic multi-systemic disorder with arthritic, gastrointestinal, mucocutaneous, ocular, vascular, and central nervous system involvement. This disease has a chronic course with periodic exacerbations and progressive deterioration (9). The etiology of BD is unclear, but viral infection has long been postulated as one of the main factors. The viral hypothesis has been verified by detection of the virus in saliva (10), intestinal ulcers (11), and genital ulcers (12) of patients with BD since Hulusi Behçet first propounded a viral aetiology (13). Furthermore, herpes simplex virus (HSV) inoculation of the earlobe of ICR mice made it possible to develop a BD-like animal model (14). Manifestations in mice after HSV inoculation involve multiple symptoms, which include oral ulcers, genital ulcers, skin ulcers, eye symptoms, gastrointestinal ulcers, arthritis, and neural involvement, as well as skin crusting. The frequency of these symptoms is similar to that of patients with BD (15). Various anti-viral drugs, including Acyclovir and Famcyclovir, have been used to treat BD, but with limited success: only 40% efficacy in BD-like mouse model compared to a 60% success rate in treating HSV (16). Moving from viral to other causes of BD, several researchers have identified lymphocyte dysfunction as a possible cause (17, 18). Attention has been focused on the T helper (Th) 1 and Th2 cytokines, with Th1 cells perhaps playing an important role in the immunopathogenesis of BD (19). When the Th2 adjuvant, aluminium hydroxide (alum), mixed with ovalbumin (OVA) was injected into mice suffering from BD, their cutaneous symptoms improved. Adoptive transfer with splenocytes from OVA-alum injected BD-like mice also resulted in an improvement. Taken together, these findings suggest that the up-regulated expression of Th2 cytokines, such as IL-4 and IL-10, induced by Th2 adjuvant can attenuate the development and improve some of the symptoms of Behçet’s disease (20). Macrophages are the major source of interleukin-4 (IL-4) during radiation-induced pneumonitis, and enhanced IL-4 production is detected in rats even 84 days after irradiation (21). IL-4 is the master cytokine of the Th2 type immune response (22). In this study, we have examined whether the radiotherapy-induced enhanced IL-4 production could improve Th1 cell-mediated inflammatory responses. To further understand the consequences, or more specifically the in vivo responses, of low-dose irradiation in immune regulation and anti-inflammation, we radiated normal, healthy and BD-like mice with chronic inflammatory symptoms. The effects of irradiation on several cytokines, chemokines, co-stimulatory molecules, inflammatory indicators and cell death markers were examined.

Materials and methods
Mice
Five-week-old male ICR mice were used in this study. To induce a BD-like disease in mice, the earlobes of the mice were scratched with a needle and then
inoculated with 1.0 x 10^6 plaque forming units/ml of HSV type 1 (F strain). Virus inoculation was performed twice with a 10-day interval, followed by 16 weeks of observation. Mice were housed in temperature- and light-controlled conventional rooms (20-22°C, 12h light cycle starting at 8:00 a.m.) and had free access to food and water. During the experiment, the animals were closely observed. Mice were handled in accordance with protocols approved by our institutional animal care committee.

**Gross observation of BD symptoms**

Manifestations in mice after HSV inoculation involved multiple symptoms, which included oral ulcers, genital ulcers, skin ulcers, eye symptoms, gastrointestinal ulcers, arthritis, and neural involvement, as well as skin crusting. Oral, genital, other skin ulcers (including bulla and crust), and eye symptoms were all classified as major symptoms. Other symptoms were classified as minor symptoms (14). Of the total number of HSV-injected mice, 15% of mice developed Behçet’s disease-like symptoms. The disappearance of symptoms and decrease in lesion size constituted improvement, similar to human patients. The time interval of observation of the animals was once per week after HSV inoculation. The severity score of Behçet’s disease was determined according to the Behçet’s disease Current Activity Form 2006 (www.behcet.ws/pdf/BehçetsDiseaseActivityForm.pdf). Among patients’ symptoms, mouth ulceration, genital ulceration, erythema, skin pustules, skin ulceration, joints-arthritis, diarrhea, red eye (right, left), reduced vision (right, left), loss of balance, discoloration, and swelling of the face were selected and analyzed in the Behçet’s disease mouse model. The score of each symptom is one and when the score was added up the total was used to determine the severity score of Behçet’s disease. Before and after treatment, the severity score was measured and compared. Mice exhibiting significantly reduced symptoms were photographed to document improvement after treatment.

**Irradiation of mice**

Normal mice were irradiated (whole- or half-body) with one of three different single doses (10cGy, 2Gy, and 10Gy) at a distance of 100 cm from source to skin using a 6 MV photon beam (LINAC 2100C/D, Varian Oncology System, Palo Alto, CA). Mice were immobilized and restrained in the supine position on a wooden board. They were irradiated through a single anterior beam. To increase the skin dosage, we placed a 5-mm thick acryl plate at a distance of 6 cm from the skin surface of mice. For half-body radiation, only the area below the xyphoid process was irradiated. The rest of the upper part of the body was shielded by the independent beam. Each dose group consisted of 40 normal mice. Sham normal mice were used as control (n=10). BD-like mice were given a single dose, either whole-body irradiated with 10 centigray (cGy) (n=7) or 2Gy (n=7) or half-body with 10cGy (n=8).

Seven BD-like mice that had not been exposed to radiation were observed for spontaneous improvements in symptoms. Normal mice were sacrificed 3 hours after irradiation or at post-irradiation day 2, 4, or 6 (10 mice per group at each time point). Peripheral blood mononuclear cells (PBMC) and sera were collected to determine the levels of cytokines, chemokines, co-stimulatory molecules, inflammatory indicators, and cell death markers as indicated using RT-PCR, FACS, and ELISA. Whole- or half-body irradiated BD-like mice that received 10cGy or 2Gy were sacrificed four days after irradiation.

**RNA isolation from PBMC and RT-PCR**

Total RNA was isolated using TRIzol™ (Gibco-BRL, MA, USA), and cDNA was prepared using Superscript™ II First-Strand Synthesis System (Gibco-BRL, MA, USA) according to the manufacturer’s instructions. The PCR was performed using PCR SuperMix (Gibco-BRL, MA, USA). The PCR primer pairs are indicated in Table I. The amplification was processed in a Perkin Elmer Thermo Cycler 900 with an initial 5 min denaturation at 94°C, followed by 30 cycles of the profile: 94°C for 30 sec; 56°C for 30 sec; and 72°C for 1 min. PCR products were separated by electrophoresis in a 0.9% agarose gel and detected under UV light.

**Flow cytometric analysis of intracellular cytokines**

Cells were incubated with Brefeldin A (5 μg/mL, Sigma) in DMEM for 3 hrs and then fixed with 4% formaldehyde and then permeabilized for 10min. PE-conjugated anti-IL-4, IFN-γ antibodies (R&D Systems Inc., Mineapolis, MN) and PE-conjugated anti-IL-10 antibodies (BD Biosciences Pharmingen, San Diego, CA) were added to the buffer. Samples were analyzed on a flow cyrometer (FACS Vantage; Becton Dickinson) with ≥20,000 gated lymphocytes. Negative isotype control was used to distinguish stained from unstained cells.

**ELISA**

Serum was analyzed for the detection of mouse IL-4, IL-10, and IFN-γ (R&D Systems Inc., Mineapolis, MN) using commercial ELISA kits. The ELISAs were carried out according to the manufacturer’s instructions. Means and standard deviations were calculated using ELISA values determined for each well. The ELISA reader was a Bio-Rad model 170-6850 microplate reader, and wavelength was 450nm.

**Statistical analysis**

All data are represented as mean ± SE. Statistical differences between the control and radiation groups were determined using the Student’s t-test and Bonferroni correction. Statistical analysis was performed using the MedCalc® version 9.3.0.0.

**Results**

Cytokine changes in PBMC and serum caused by whole-body and half-body irradiation

Low-dose (10cGy), medium-dose (2Gy) and high-dose (10Gy) radiation were delivered to whole-body or half-body of individual normal mice, and PBMC cytokine mRNA levels were compared after exposure. The time-course and dose-response effects of whole-body and half-body (lower
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Body irradiation are shown in Fig. 1A and 1B, respectively. IFN-γ mRNA and IL-12p40 mRNA were present in the PBMC from untreated normal mice, while PBMC cytokine mRNA for IL-4, IL-10 and tumor necrosis factor (TNF-α) was detected in normal mice following whole-body irradiation. IL-4 levels increased two days and peaked four days after 10cGy and 2Gy irradiation. IL-10 mRNA was detected four days after 2Gy irradiation. TNF-α mRNA was detected at 3 hrs of the treatment and peaked four days after 10cGy and 2Gy irradiation. PBMC from mice receiving 10 Gy irradiation expressed IFN-γ and IL-4 at day 2. Half-body irradiation induced IL-4, IL-12p40 and TNF-α expression patterns similar to those observed following whole-body irradiation with 10cGy and 2Gy (Fig. 1B). On the other hand, the increase of IL-4 and TNF-α following half-body irradiation with 10Gy showed a different pattern from that observed after whole body irradiation.

IL-4 and IL-10, which are representative Th2 cytokines, have been shown to attenuate the symptoms of BD (20), while IFN-γ is the key cytokine produced by Th1 cells (23). On the basis of these studies, the protein levels of IL-4, IL-10 and IFN-γ were measured. Following 10 cGy whole-body irradiation, protein levels were determined using FACS and ELISA (Fig. 2A). FACS analysis indicated that the three cytokines all reached peak levels four days after exposure. The percentage of IL-4-positive cells in the whole PBMC sample, as determined by cytofluorimetric analysis, at day four (7.24±0.92%) was greater than that for control mice (1.3±0.1%) (p<0.005). IL-10 (7.92±0.67%) and IFN-γ (17.62±6.65%) production was also increased at day four compared to control mice (1.50±0.13% p<0.05; 7.62±0.66% p<0.05, respectively). Serum IL-4 peaked six days after treatment, while IL-10 and IFN-γ peaked at day four. IL-4 levels increased from 1.8±0.8 to 6.08±1.7 pg/ml (p<0.05) after the 10 cGy whole-body irradiation, while IL-10 levels increased from 73.36±6.14 to 153.13±18.20 pg/ml (p<0.05), and IFN-γ increased from 34.27±6.70 to 102.34±12.00 pg/ml.

Fig. 1. Cytokine expression in PBMC from normal mice treated with 10cGy, 2Gy, 10Gy irradiation, compared to untreated normal mice: RT-PCR. The number of mice for each time point in each dose group and control group was 10. A. Whole-body irradiation B. Half-body irradiation.

Fig. 2. A. FACS and ELISA analyses of PBMC and serum from 10Gy whole-body irradiated mice compared to untreated normal mice. For each time point, the number of mice used was 10, 5 being for FACS study and 5 for the ELISA. B. IL-4/IFN-gamma ratio in individual samples was calculated. The ratios in irradiated groups were statistically significantly increased and compared to control group in FACS and ELISA.
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(p < 0.05) after the irradiation. Th2/Th1 balance by IL-4/IFN-γ ratio in individual samples was calculated (Fig. 2B). FACS and ELISA analysis revealed that the ratios in irradiated groups were increased and statistically significant compared to the control group.

**Half-body, low-dose (10cGy) irradiation increased serum cytokine levels in normal mice:**

**ELISA experiments**

Serum levels of the cytokines IL-4, IL-10 and IFN-γ from whole-body and half-body irradiated mice were compared at 3 hrs, 2, 4, and 6 days. Figure 3 shows that half-body irradiation resulted in higher protein levels than whole-body treatment: IL-4 levels of 8.66±1.80 pg/ml and 11.18±0.80 pg/ml at days 4 and 6, respectively, while whole-body irradiation resulted in 3.32±1.10 pg/ml (day 4; p < 0.05) and 6.08±1.70 pg/ml (day 6; p < 0.05). At day 6, the serum of half-body irradiated mice had IL-10 levels of 219.31±15.10 pg/ml compared to 130.20±4.20 pg/ml in whole-body irradiated mice (p < 0.005). Half-body irradiation yielded IFN-γ sera levels of 349.22±39.80 pg/ml, while whole-body irradiation resulted in 102.34 ± 24.00 pg/ml (p < 0.0005) four days after treatment. Half-body irradiation was, therefore, more effective than whole-body irradiation in increasing serum IL-4, IL-10 and IFN-γ levels.

**Chemokine levels in normal mice are altered after irradiation:**

**RT-PCR**

Four days after treatment with 10cGy or 2Gy irradiation, mice were sacrificed and PBMC were collected to assess chemokine expression. Induction of IL-18, RANTES (regulated on activation, normal T cell expressed and secreted), TGF-β, interferon-inducible protein (IP)-10, macrophage inhibitory protein (MIP)-1α, monocyte chemotactic protein (MCP)-1, cyclooxygenase (COX)-2, Fas, and lymphotactin (LTP) mRNA synthesis by whole- or half-body irradiation with 10cGy and 2Gy was compared using RT-PCR. Relative to normal controls, the level of RANTES mRNA was increased following whole-body and half-body treatment. The synthesis of perforin mRNA was induced by 2Gy whole-body irradiation, and 10cGy and 2Gy half-body treatments. MCP-1 mRNA synthesis was induced by whole-body 10cGy and 2Gy treatments. Inducible nitric oxide synthase (iNOS) was induced by only 2Gy whole-body treatment (Fig. 4). Overall, low-dose and medium-dose irradiation of normal mice increased the expression of most chemokines examined in this experiment.

**Irradiation affects IL-4 production in BD-like mice**

Whole-body (10cGy, 2Gy) and half-body (10cGy) irradiation was applied to BD-like mice to examine the effects of chemokine regulation and anti-inflammatory properties (Fig. 5B) of irradiation. On the fourth day after irradiation, mice were sacrificed and PBMC was collected for RT-PCR. Expression of MIP-1α, a factor that is known to reduce BD-like symptoms (18), was induced by 10cGy and 2Gy whole-body and 10cGy half-body irradiation. Levels of IP-10, a known attractant of peripheral blood lymphocytes, and LPT, a key modulator of T cell trafficking in the pathogenesis of rheumatoid arthritis (RA), were both reduced following 10cGy half-body irradiation. The representative cytokines of Th2 cells, IL-4 and IL-10, and Th1 cells, IFN-γ were...
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PBMC chemokine expression was measured by RT-PCR after 10cGy, 2Gy whole-body and half-body irradiation and compared to untreated normal mice. Data was obtained from 10 mice in each case.

Mucocutaneous symptoms of BD-like mice were improved after 10cGy half-body irradiation

BD-like mice were photographed four days after 10cGy half-body irradiation. Improved skin lesions (Fig. 6), in seven of the eight (87.5%) treated mice, included earlobe inflammation, skin ulcers on the face, back, foreleg, and scruff, and genital ulcers. For the eighth mouse, no change in large skin ulcer (greater than 1cm in diameter) was observed.

The symptoms of 10cGy whole-body irradiated BD-like mice were improved in four of the seven (57.1%) treated mice, and the symptoms which improved were similar to those that responded to half-body irradiation. Mucocutaneous ulcerations in 2Gy whole-body irradiated BD-like mice were improved in two of the seven (28.6%) treated mice. The symptoms of the five remaining mice were not changed by irradiation.

For anti-inflammatory effects, low-dose 10cGy half-body irradiation was the

Fig. 4. PBMC chemokine expression was measured by RT-PCR after 10cGy, 2Gy whole-body and half-body irradiation and compared to untreated normal mice. Data was obtained from 10 mice in each case.

Fig. 5. Effects of 10cGy irradiation on BD-like mice. A. Cytokines in half-body irradiation (Lane 1,2,3,4: BD mouse number 1,2,3,4). B. Cytokines in whole-body irradiation (Lane 1,2,3,4: BD mouse number 1,2,3,4). C. Chemokines (lane 1: BD non-irradiated, lane 2: BD, 10cGy whole-body irradiated, lane 3: BD, 2Gy whole-body irradiated, lane4: BD, 10cGy half-body irradiated).
most effective in reducing symptoms in BD-like mice. Seven BD-like control mice that were not treated with radiation showed no signs of improvement of their symptoms during the period of the experiment.

**Discussion**

Th1 cells have been reported to be selectively reduced after total lymphoid irradiation (24) and suppressor T cell-activating macrophages induce TGF-β-dependent form of T cells in ultraviolet-irradiated human skin (25). Behçet’s disease is a Th1 cytokine polarized disease, therefore, we expected that irradiation could evoke Th2 cytokine and that evoked Th2 cytokine can balance upregulated Th1 cytokine and this balance can have a positive influence on Th1 polarized BD patients. This study was designed to evaluate the potential therapeutic effectiveness of low-dose irradiation on chronic inflammatory disease in a mouse model of Behçet’s disease. Knowledge from this work could be applied to the treatment of chronic inflammatory diseases and may particularly be applicable to patients with drug resistance and liver problems. Numerous mechanisms have been suggested for the clinically observed effects of irradiation on benign diseases. However, only a few experiments have been reported. Therefore, we explored the effects of low-dose irradiation on both normal mice and a BD-like mouse model induced by herpes simplex virus, so that they have the highest clinical resemblance to the human disease (26).

In the present study, the serum levels of IL-4, IL-10, and IFN-γ were higher in mice subjected to half-body irradiation compared to whole-body irradiation. Chemokine expression in PBMC was also found to be anti-inflammatory regulated following half-body irradiation. On this basis, we chose to apply half- and whole-body irradiation to BD-like mice and to examine cytokine expression and test for improvement of symptoms. In BD-like mice, 10cGy half-body irradiation was more efficient than whole-body to improving the BD symptoms (87.5% vs. 57.1%). High doses of irradiation were not further examined on the BD-like model since cytokine expression was lower than that with low/medium doses.

We focused on changes of cytokine and chemokine expression in PBMC following low-dose irradiation and RT-PCR revealed that IL-4, IL-10, IL-12p40, TNF-α, TGF-β, IP-10, MIP-1α, MCP-1, LPT, IL-6, Fas, perforin, and iNOS expressions were increased in irradiated normal mice compared to non-irradiated mice. It has been previously reported that Th2 cytokines, such as IL-4 and IL-10, can attenuate the symptoms of BD (20). Since IFN-γ is the key cytokine produced by Th1 cells and is an important immunoregulatory factor (27), the protein levels of IL-4, IL-10 and IFN-γ were measured, and FACS and ELISA analyses confirmed increased IL-4, IL-10, and IFN-γ protein levels in PBMC and sera. IL-4/IFN-gamma ratios were statistically significantly increased after irradiation.

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**Fig. 6.** 10cGy half-body irradiation improved the symptoms of BD-like mice on the fourth day of treatment.

**Fig. 7.** The comparison of the severity score before and 2 weeks later after irradiation to BD-like mice.
compared to the non irradiated control group. It should be mentioned that modulation of the cytokines IL-4 and IFN-γ in PBMC following in vitro low-dose irradiation has previously been described (28). Furthermore, splenocyte IL-10 synthesis has been shown to increase by irradiation (29) and increased IL-10 levels help ameliorate mucocutaneous symptoms of BD-like mice (17).

TNF-α expression in splenocytes is known to increase after 0.2Gy total-body irradiation (30), and TNF-α mRNA levels in human peripheral blood T lymphocytes have been shown to increase in response to irradiation (31). However, it has also been reported that inhibition of TNF-pathway with infliximab improves the symptoms of therapy-resistant chronic inflammatory disorders (32). In the present study, low-dose irradiation increased TNF-α mRNA levels in this experiment, BD-like symptoms improved. The increase in TNF-α mRNA levels was overcome by the increase of IL-4 (33), Th2/Th1 ratio, and in other cytokines and chemokines.

IFN-γ (34) and IL-12p40-35 induced activation of resident peritoneal macrophages by gamma-irradiation was confirmed. Ohno et al. reported lower IFN-γ levels in patients with active, compared to inactive BD (36). IL-12 levels have also been shown to correlate with disease activity (37, 38). IL-12 consists of two disulfide-linked subunits, p40 and p35, that form functionally active heterodimers. IL-12p40 monomers and homodimers have inhibitory effects on Th1 cells, leading to a Th2 environment (39). Increased IL-12p40 levels may also contribute to the creation of the Th2-type environment, possibly leading to a reduction in the severity of BD symptoms.

Increased TGF-beta levels contribute to a reduction of endothelial cell adhesion to mononuclear cells following low-dose irradiation (8). Down-regulation of leukocyte/PBMC adhesion to endothelial cells may contribute to the anti-inflammatory effects of low-dose irradiation. TGF-beta is also known to induce regulatory T cells (40) and regulatory T cells decrease inflammation by the balance between T cell immunity and tolerance in the mucosal system (41). Low-dose gamma-ray irradiation suppresses inflammation of collagen-induced arthritis in MRL-1pr/lpr mice. The population of CD4+CD25+ regulatory T cells has been shown to be significantly increased and pro-inflammatory cytokines are suppressed in irradiated mice (42, 43).

Fas is a cell-surface protein that belongs to the tumor necrosis factor receptor family. Irradiation has been shown to up-regulate Fas in a dose-dependent manner (44), and thalidomide treatment increases Fas protein levels in mouse spleens (17). Fas system dysregulation leads to uncontrolled lympho-proliferation, indicating that the Fas/FasL system plays a central role in immune reaction control via cell death activation (45, 46).

Low-dose radiotherapy of arthritic joints, applied during the acute inflammatory response phase, improves the clinical and histomorphological symptoms of adjuvant arthritis (47). However, low-dose irradiation in the present study did not influence iNOS levels in BD-like mice, when examined by RTPCR. Orem et al. reported decreased NO production in patients with Behçet’s disease (48), whereas Yildirim et al. reported higher serum levels of NO metabolite in patients with active BD patients, compared with patients with inactive BD (49). Also, there was no significant difference in the frequencies of inducible NO synthase gene polymorphisms between patients with BD and control subjects (50). Therefore, the relationship between BD and NO remains controversial.

Increases of IP-10, MIP-1α, MCP-1, LPT and perforin expression levels in response to low-dose irradiation have not previously been reported. IP-10 is a chemokine that recruits activated T lymphocytes in the liver during chronic HCV infection (51). In our experiments, low- and medium-dose, whole- and half-body irradiation increased PBMC IP-10 mRNA levels in normal mice, however low-dose half-body irradiation did not increase IP-10 mRNA levels in BD-like mice. Interestingly, normal and BD-like mice showed different IP-10 expressions after low-dose half-body irradiation. Decreased IP-10 levels in BD-like mice could negatively influence peripheral blood lymphocyte attraction, thereby improving BD-symptoms. The chemokine MIP-1α has also been reported to be differently regulated in normal and BD-like mice. MIP-1α was up regulated in thalidomide-treated BD-like mice, however not expressed in thalidomide treated normal mice (18). MIP-1α maintains haemopoietic recovery in vivo following repeated sublethal irradiation cycles, and reduces accumulated haemopoietic stem cell damage following repeated non-cell cycle-specific cytotoxic insults (52).

LPT is known to be a key T-cell trafficking modulator in pathogenesis of rheumatoid arthritis (RA) and has immunomodulatory effects (53). LTP increases also during radiation-induced pneumonitis and fibrosis development (54). In the present study, low-dose irradiation decreased LTP levels in BD-like mice.

In summary, we have confirmed here that low-dose irradiation of normal and BD-like mice increases immune cell cytokine and chemokine secretion. In addition, low-dose, half-body irradiation of BD-like mice improved their dermatitis symptoms by over 80%. Our results suggest that low-dose irradiation could be effective in the treatment of chronic inflammatory diseases, particularly in patients who are either unresponsive to drug treatments or have severe liver problems.

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