Effects of various radiation doses on induced T-helper cell differentiation and related cytokine secretion

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ABSTRACT

Exposure to ionizing radiation often induces T helper (Th) cell differentiation, resulting in an imbalance of Th1 and Th2 cellular subtypes, which can affect the efficacy of cancer radiotherapy. The aim of this study was to analyze differential expression of Th1, Th2 and Th3/Type 1 regulatory T cell (Tr1) subtype–related genes and cytokines in mouse thymocytes after high- and low-dose systemic radiation, using functional classification gene arrays and Elisa assays, and to explore the molecular mechanisms underlying radiation’s immune effects and their relationship with Th1/Th2 immunity. We found that expression of 8 genes was upregulated after LDR, while expression of 5 genes was downregulated. After HDR, 54 genes were upregulated and 3 genes were downregulated, including genes related to Th1, Th2 and Th3/Tr1 cellular subtypes, Th1/Th2-type immune response genes and transcription factor–related genes. In the foregoing results, LDR and HDR in the thymus induced opposite patterns of expression for Th1-, Th2- and Th3-type related cytokines TGF-β, C/EBP-β and TNF-α. We also found that expression of Interferon-γ (IFN-γ) and Interleukin-2 (IL-2), which have a moderating effect on immune function, was upregulated after LDR. Furthermore, the secretion of negative regulatory factors Interleukin-1β (IL-1β), Interleukin-4 (IL-4), transforming growth factor-β (TGF-β) and Interleukin-21 (IL-21) was reduced after LDR, but HDR produced the opposite effect and stimulated their expression. These findings suggest that LDR may induce a Th1-type immune response, while HDR may lead to a Th2-type immune response.

Keywords: radiation; dose; Th cells; PCR array; immune response

INTRODUCTION

In recent years, immunological theory has proposed that T helper (Th) cells can be classified according to their secretion of cytokines and that Th functions can be divided into different subgroups composed of Th1, Th2 and Th3/Type 1 regulatory T (Tr1) cells. Th1 cells mainly secrete IL-2 and IFN-γ, enhance the toxic effects of killer T cells, promote macrophage activation and participate in the regulation of cellular immunity, which facilitates the differentiation of cytotoxic T cells and mediates the cellular immune hypersensitive response. Th2 cells secrete IL-4, IL-6 and IL-10, which mediate humoral immunity and promote antibody production [1, 2]. Th3/Tr1 cells secrete TGF-β, which exerts a negative regulatory effect on immune responses. The balance of Th1/Th2 cells is important for maintaining immune health of the host [3]. Th1 cells promote resistance to intracellular pathogens and secrete cytokines such as IFN-γ, a major cytokine associated with host resistance to viral infections. Th2 cells promote resistance to extracellular pathogens and importantly to the current study, antagonize the production and activity of Th1 cytokines, including IFN-γ [4, 5]. Thus, an altered Th1/Th2 balance can influence host susceptibility to a
variety of immune-mediated diseases, including allergy, autoimmunity and increased infections [6, 7]. Exposure to ionizing radiation often reduces Th1 function, resulting in a Th1/Th2 imbalance that may affect the efficacy of cancer radiotherapy [8, 9]. It has been reported that ionizing radiation induced a lasting Th2 immune response while depressing Th1 immune responses in experimental animals and atomic bomb survivors. Th1/Th2 imbalance also accounts for radiation-induced immunosuppression [10–12]. A large number of studies have confirmed that low-dose radiation (LDR, 0.075–0.2 Gy) and high-dose radiation (HDR, 2.0–6.0 Gy) induce different biological effects that can stimulate organism or tissue adaptive functions, expressed as a series of adaptive reactions [13], activation of immune function [14, 15] and prevention and treatment of inflammatory diseases [16], in which the activation of T lymphocytes plays a central role in the radiation-induced immune effect. In this study, we used Th1-Th2-Th3 functional classification arrays to detect and analyze HDR- and LDR-induced differential expression of thymus Th cell subtype function-related genes, and enzyme-linked immunosorbent assay (ELISA) was used to verify the expression of cytokines related to immune functional regulation, in order to explore the molecular mechanisms underlying the effect of different radiation doses on immune function.

MATERIALS AND METHODS

Experimental animals and groups
A total of 16 healthy ICR mice, half male and half female, 6 to 8-week-old, weighing 18–22 g, were purchased from Jilin University Bethune Medical Laboratory Animal Center (Changchun, China), production license number SCXK-Kyrgyzstan 2008-0005 and use of permits lot number SCXK (Jilin) 2008-0011. Mice were housed in individual cages in a temperature-controlled room at 22 ± 2°C with relative humidity of 50 ± 10% and a 12 h dark/light cycle. Food and water were provided ad libitum throughout the experiment. Mice were irradiated in a holder designed to immobilize unanesthetized mice to ensure the accuracy of radiation doses. The mice were randomly divided into three groups: control group (n = 4), LDR group (n = 4) and HDR group (n = 8).

Irradiation conditions
Irradiation was performed with an X-ray fixed deep treatment machine X.S.S.205 FZ (Dandong, China). HDR group mice were whole-body irradiated at a single high dose (2.0 Gy) of X-rays administered from a source–skin distance of 60 cm, at the dose rate of 343 mGy/min. LDR group mice were whole-body irradiated at a single low dose (0.075 Gy) of X-rays administered from a source–skin distance of 178.50 cm, at a dose rate of 12.5 mGy/min.

Tissue collection
The mice were euthanized 24 h after X-ray irradiation, and the thymus tissue of each mice was individually labeled and collected in Eppendorf tubes then stored in liquid nitrogen. Dry ice was used the next day to transport the tissues to the test facility (KangCheng Biological, Shanghai, China). The tissues were homogenized using Teflon tissue homogenizers (BILON-08; Shanghai Bilon Instrument Manufacturing Co. Ltd, Shanghai, China) as recommended by the manufacturer. The resulting single-cell suspensions were centrifuged for 10 min at 12 000 r/min and the cytokines levels were measured in the homogenates by ELISA (all from R&D Systems).

RNA preparation
Total RNA was isolated from tissue samples with the use of TRIZOLâ® Reagent (Invitrogen,USA) and an RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions. The RNA was treated with an RNase-free DNase I Kit (Qiagen) to remove contaminating genomic DNA. RNA quality was checked with 1% agarose gel electrophoresis, and RNA integrity was analyzed with a 2100 Bioanalyzer (Agilent Technologies, USA). RNA quantity was determined with the ND-1000 Spectrophotometer (Nanodrop Technology, Wilmington, DE). Samples with 260/280 nm absorbance ratio of >1.8–2.0 were used for further experiments.

Gene profiling microarray
Total RNA (1 µg) was reverse transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen). Gene expression was analyzed with the Mouse Th1-Th2-Th3 RT² Profiler™ PCR Array (Qiagen). cDNA was amplified in the presence of 84 specific primers coated in 96-well microtiter plates on a CFX96 Real-Time System (Bio-Rad, Munich, Germany). The 84 evaluated genes are associated with T cell–derived cytokines, CD4+ T cell markers, the T regulatory (Treg) cell network, T cell activation and relevant transcription factors. Samples were normalized to GAPDH as the reference gene. CFX Manager software (Bio-Rad, USA) was used to view the amplification of each gene and corresponding Ct values.

Enzyme-linked immunosorbent assay
ELISA was used to quantify concentrations of Th1-Th2-Th3-related cytokines IFNγ, IL-2, IL-1β, IL-4, TGF-β and IL-21 in each group. ELISA kits for the detection of each cytokine were obtained from R&D (R&D Systems, Minneapolis, MN). The assays were performed in duplicate with 50 µl of sample added to each well following the manufacturer’s instructions. The readings were taken in an Epoch BioTek® ELX 800 plate reader (BioTek, Winooski, VT). The OD was read at 450 nm with reference to 630 nm. A standard curve was prepared for each cytokine, and the corresponding curve formulas were used to calculate the sample concentrations.

Statistical analysis
The relative expression of each gene in the two dose groups was calculated by the $2^{-\Delta\Delta Ct}$ method, in which Ct indicates the fractional cycle number when the fluorescent signal reaches detection threshold. The relative amount of the control was calculated and compared with the relative amount of the sample (quantitative ratio). The ratio of the irradiated group to the sham group was $>2.0$, and the decrease was <0.5 [17]. The data for quantitative gene expression were characterized using descriptive statistics, and the statistical significance of differences in gene and cytokine expression between groups was calculated using Student’s t-test. All statistical tests were
performed with SPSS version 22.0 (SPSS, Inc., Chicago, USA). \( P < 0.05 \) was considered as a statistically significant difference.

**RESULTS**

**Gene expression profile of Th1-Th2-Th3 cytokine-related genes after various doses of radiation**

The expression of differentially expressed genes in the Th1-Th2-Th3 RT2 Profiler™ PCR Array was analyzed. The LDR group had 13 differentially expressed genes compared with the control group, while the HDR group had 57 differentially expressed genes compared with controls. Among the differentially expressed genes, 8 genes were significantly upregulated in mice thymocytes after LDR and 5 genes were significantly downregulated (\( P < 0.05 \)). After HDR, 54 genes were upregulated in thymocytes and 3 genes were downregulated (Fig. 1).

**LDR-induced Th1-Th2-Th3-related gene functional analysis**

Table 1 shows that LDR induced upregulation of Th1 cell–related genes Stat4 and Socs1, downregulation of Th2 and Th3/Tr type cell–associated genes Il-4ra, Cebpb, Gata3 and Tgfb3 and upregulation of the Th1-type immune response gene Sftpd.
HDR-induced Th1-Th2-Th3-related gene functional analysis

A 2.0 Gy dose of radiation induced upregulation of Th1 type, Th2 type and Th3/Tr type cell–related genes, but had no effect on Th1-type immune response–related gene expression (Table 2). In contrast, Th2-type immune response–related genes Cd86, Il-18, Il-10 and Irf4 were significantly upregulated.

**Analysis of transcription factors and related genes in Th1-Th2-Th3 cells induced by various doses of radiation**

LDR induced upregulation of Stat4 and downregulation of Cebpb and Gata3 (Table 3). HDR induced significant upregulation of transcription factor–related genes Tbx21, Stat1, Stat4, Irf1, Nfkb1 and Cebpb, but no genes were significantly downregulated by HDR.

**LDR and HDR induced opposing effects on Th1-Th2-Th3-related genes**

Fig. 2 shows that after high and low doses of X-ray irradiation in mice, the thymus cells expressed opposing patterns of response for three Th cell–related genes. TGF-β, C/EBP-β and TNFα were significantly upregulated more than 2-fold after HDR irradiation, but were downregulated after LDR irradiation, with the decrease in TGF-β expression being >4-fold (P < 0.05).

**Table 1. LDR-induced differential expression of Th1-Th2-Th3-related genes in the thymus**

| Category                        | Gene symbol | Gene name                                | Fold change | P value |
|---------------------------------|-------------|------------------------------------------|-------------|---------|
| Th1 cell–related genes          |             |                                          |             |         |
| Upregulated                     | Stat4       | Signal transduction and transcriptional activator 4 | 2.97        | 0.0000  |
|                                  | Socs1       | Suppressor of cytokine signaling 1        | 2.22        | 0.0012  |
| Downregulated                   | Tnf         | Tumor necrosis factor                     | −2.09       | 0.0025  |
| Th2 cell–related genes          |             |                                          |             |         |
| Upregulated                     | Il-4Ra      | Interleukin 4 receptor, α                 | −3.25       | 0.0018  |
|                                  | Cebpb       | cAMP reaction element binding protein     | −3.02       | 0.0034  |
|                                  | Gata3       | GATA binding protein 3                    | −2.20       | 0.0213  |
| Th3/Tr1 cell–related genes      |             |                                          |             |         |
| Upregulated                     | none        |                                          |             |         |
| Downregulated                   | Tgfb3       | Transforming growth factor, beta 3         | −4.03       | 0.0012  |
| Th1 type immune response genes  |             |                                          |             |         |
| Upregulated                     | Sftpd       | Surfactant-related protein D              | 2.54        | 0.0018  |
| Downregulated                   | none        |                                          |             |         |

**HDR-induced Th1-Th2-Th3-related gene functional analysis**

**Table 2. HDR-induced differential expression of Th1-Th2-Th3-related genes in the thymus**

| Category                        | Gene symbol | Gene name                                | Fold change | P value |
|---------------------------------|-------------|------------------------------------------|-------------|---------|
| Th1 cell–related genes          |             |                                          |             |         |
| Upregulated                     | none        |                                          |             |         |
| Downregulated                   | Il-1β       | Interleukin 1                            | −4.03       | 0.0012  |
| Th2 cell–related genes          |             |                                          |             |         |
| Upregulated                     | none        |                                          |             |         |
| Downregulated                   | Il-4Rα      | Interleukin 4 receptor, α                 | −3.25       | 0.0018  |
|                                  | Cebpb       | cAMP reaction element binding protein     | −3.02       | 0.0034  |
|                                  | Gata3       | GATA binding protein 3                    | −2.20       | 0.0213  |
| Th3/Tr1 cell–related genes      |             |                                          |             |         |
| Upregulated                     | none        |                                          |             |         |
| Downregulated                   | Tgfb3       | Transforming growth factor, beta 3         | −4.03       | 0.0012  |
| Th1 type immune response genes  |             |                                          |             |         |
| Upregulated                     | Sftpd       | Surfactant-related protein D              | 2.54        | 0.0018  |
| Downregulated                   | none        |                                          |             |         |

**Th1-Th2-Th3-related cytokine expression in mouse thymocytes after various doses of radiation**

In final experiments, we determined the effect of LDR and HDR on cytokine secretion from Th1-Th2-Th3 cells in the mouse thymus. Figure 3 shows that IFN-γ and IL-2 secretion were significantly upregulated after LDR (P < 0.05), while IL-4 (P < 0.05), IL-1β, IL-21 and TGF-β (P < 0.01) were downregulated. In contrast, regulation of cytokine secretion after HDR showed the opposite pattern.

**DISCUSSION**

The thymus is an important central immune organ for T lymphocyte differentiation and maturation, and is one of the target organs highly sensitive to ionizing radiation. Therefore, the radiation response of thymocytes is an important focus of radiation immunology research. Different doses of ionizing radiation can produce different biological effects on the body. Higher doses of irradiation have a detrimental effect on the body, while lower doses of ionizing radiation may have the opposite effect. The United Nations Atomic Radiation Effects Scientific Committee reported in 1986 that the level of radiation should be considered low when >0.05 Gy of high–Linear Energy Transfer (LET) radiation or <0.2 Gy of low-LET radiation, while radiation dose rates should be <0.05 mGy/min to be considered low. A large number of experimental studies have confirmed that low doses of ionizing radiation can stimulate immune function [18, 19].
Table 2. HDR-induced differential expression of Th1-Th2-Th3-related genes in the thymus

| Category                      | Gene symbol | Gene name                           | Fold change | P value |
|-------------------------------|-------------|-------------------------------------|-------------|---------|
| **Th1 cell-related genes**    |             |                                     |             |         |
| Upregulated                   | *Ifng*      | Interferon γ                         | 13.14       | 0.0022  |
|                               | *Il-2ra*    | Interleukin 2 receptor, α            | 13.00       | 0.0052  |
|                               | *Ccr5*      | Chemokine receptor 5                 | 9.51        | 0.0024  |
|                               | *Il-18bp*   | Interleukin 18 binding protein       | 8.24        | 0.0324  |
|                               | *Tbx21*     | T-box 21                            | 7.68        | 0.0003  |
|                               | *Stat1*     | Signal transduction and transcriptional activator 1 | 5.96 | 0.0007 |
|                               | *Il-18*     | Interleukin 18                        | 5.63        | 0.0109  |
|                               | *Stat4*     | Signal transduction and transcriptional activator 4 | 5.61        | 0.0001  |
|                               | *Igsf6*     | Immunoglobulin superfamily member 6  | 5.34        | 0.0032  |
|                               | *Il-12b*    | Interleukin 12B                      | 4.83        | 0.0042  |
|                               | *Irf1*      | Interferon regulatory factor 1        | 4.28        | 0.0458  |
|                               | *Socs1*     | Suppressor of cytokine signaling 1    | 3.03        | 0.0005  |
|                               | *Tnf*       | Tumor necrosis factor                | 3.00        | 0.0053  |
|                               | *Socs5*     | Suppressor of cytokine signaling 5    | 2.89        | 0.0074  |
|                               | *Il-18r1*   | Interleukin 18 receptor 1             | 2.77        | 0.0033  |
|                               | *Csf2*      | Colony stimulating factor 2           | 2.07        | 0.0013  |
|                               | *Il-2*      | Interleukin 2                         | 2.06        | 0.0002  |
| Downregulated                 | none        |                                     |             |         |
| **Th2 cell-related genes**    |             |                                     |             |         |
| Upregulated                   | *Ccl7*      | Chemokine ligand 7                   | 11.14       | 0.0034  |
|                               | *Il-5*      | Interleukin 5                        | 8.74        | 0.0003  |
|                               | *Ccl5*      | Chemokine ligand 5                   | 7.17        | 0.0098  |
|                               | *Il-13Ra1*  | Interleukin 13 receptor, α1           | 5.88        | 0.0061  |
|                               | *Il-10*     | Interleukin 10                       | 5.27        | 0.0011  |
|                               | *Ccr3*      | Chemokine receptor 3                 | 4.87        | 0.0101  |
|                               | *Tlr4*      | Toll receptor 4                      | 4.06        | 0.0021  |
|                               | *Maf*       | Transonic fibrosarcoma AS42 oncogene homologues | 3.11 | 0.0126 |
|                               | *Irf4*      | Interferon regulatory factor 4        | 2.80        | 0.0028  |
|                               | *Cehp1b*    | cAMP reaction element binding protein | 2.51 | 0.0103 |
|                               | *Ccl11*     | Chemokine ligand 11                  | 2.18        | 0.0316  |
|                               | *Jak1*      | Janus kinase 1                       | 2.02        | 0.0027  |

Continued
Table 2. Continued

| Category          | Gene symbol | Gene name                                | Fold change | P value |
|-------------------|-------------|------------------------------------------|-------------|---------|
| Downregulated     | Il-4ra      | Interleukin receptor 4, α                | −5.17       | 0.0059  |
|                   | Ccr4        | Chemokine receptor 4                     | −2.79       | 0.0165  |
|                   | Gfi1        | Independent growth factor 1              | −2.02       | 0.0041  |
| Th3/Tr1 cell-related genes |           |                                          |             |         |
| Upregulated       | Il-23a      | Interleukin 23, α                        | 4.03        | 0.0015  |
|                   | Il-17a      | Interleukin 17A                          | 2.18        | 0.0001  |
|                   | Tgfb3       | Transforming growth factor, β3           | 2.18        | 0.0012  |
| Downregulated     | none        |                                          |             |         |
| Th2 type immune response genes |           |                                          |             |         |
| Upregulated       | Cd86        | CD86 antigen                             | 7.26        | 0.0096  |
|                   | Il-18       | Interleukin 18                           | 5.63        | 0.0109  |
|                   | Il-10       | Interleukin 10                           | 5.27        | 0.0011  |
|                   | Ifn4        | Interferon regulatory factor 4           | 2.80        | 0.0028  |
| Downregulated     | none        |                                          |             |         |

Table 3. Effects of LDR and HDR on Th1-Th2-Th3 cell-related transcription factors in mouse thymus

| Features | Gene symbol | Brief description of genes                                           | Fold change | P value |
|----------|-------------|---------------------------------------------------------------------|-------------|---------|
| LDR      |             |                                                                     |             |         |
| Upregulated | Stat4  | Signal transduction and transcriptional activator 4                | 2.97        | 0.0001  |
| Downregulated | Cebpb | cAMP reaction element binding protein                              | −3.02       | 0.0034  |
| HDR      |             |                                                                     |             |         |
| Upregulated | Thbx21 | T-box 21                                                             | 7.68        | 0.0003  |
|           | Stat1      | Signal transduction and transcriptional activator 1                | 5.96        | 0.0007  |
|           | Stat4      | Signal transduction and transcriptional activator 4                | 5.61        | 0.0001  |
|           | Ifn1       | Interferon regulatory factor 1                                      | 4.28        | 0.0458  |
|           | Nfkb1      | The light peptide gene enhances the nuclear factor 1, p105          | 2.79        | 0.0292  |
|           | Cebpb      | cAMP reaction element binding protein                               | 2.51        | 0.0103  |
| Downregulated | none |                                                                     |             |         |
The functional classification chip, because of its strong targeting, avoids the interference of the irrelevant genes in the spectrum chip, and has high accuracy and sensitivity. Therefore, it has many applications in the study of radiation immunology. The latest development is the PCR functional classification chip, using real-time quantitative PCR technology and gene chip technology to provide accurate quantitative detection of mRNA expression levels for hundreds of genes on the same chip [20]. The chip technique overcomes the shortcomings of the previously applied differential display PCR method, including high false positivity rates, poor reproducibility and bias for high-copy genes. T helper (Th) cell subtypes Th1 and Th2 originate from common naïve precursor cells in response to antigen and cytokine stimulation. Th cells are functionally divided according to their cytokine profiles and types of mediated immune reaction. It is well recognized that disturbances in the balance between Th1 and Th2 responses can promote immune-mediated diseases. An enhanced Th2 response is involved in atopic diseases such as asthma, whereas a dominating Th1 response is implicated in chronic autoimmune diseases like type 1 diabetes and rheumatoid arthritis.

In this study, the results using a Th1-Th2-Th3 functional sorting chip showed that LDR mainly upregulated Stat4 in Th1 cells and downregulated the key transcription factor Gata3 in Th2 cells; meanwhile, expression of TGF-β was strongly suppressed in Th3/Tr1 cells. TGF-β has now been determined to be the sole factor exerting anti-proliferative effects on Th1 and Th2 cells that eventually lead to the Th3 immune response, by perturbing the differentiation of either Th1 or Th2 cells and inhibiting proinflammatory cytokine production [21]. It has been reported that IFN-γ/IL-4 and T-bet/GATA3 play a role in the regulation of Th1/Th2 immune function [22]. The finding by Gridley et al. that alterations in CD4+ T cell gene expression, although strikingly different in 0.01 Gy- and 0.10 Gy-exposed cells, supports a shift in the Th1/Th2 balance toward the Th1 phenotype and indicates that LDR radiation promotes Th1 cell differentiation. This suggests that cell-mediated responses against virus-infected and other aberrant cells can be enhanced [23], which may induce Th1 immune responses and ultimately improve immune function. Recent studies have shown that IL-12 can differentiate CD4+ cells into Th1 cells via the Stat4 signaling pathway, whereas IL-4 induces the differentiation of naïve Th0 cells into Th2 cells through the Stat6 signaling pathway. The polarization of Th1/Th2 cells is related to the expression of different Socs members [24]. Socs proteins are negative regulators of the Jak–Stat signal transduction pathway that is triggered by many cytokine–receptor interactions [25]. The expression of Socs1 was twice as high when Th cells were differentiated into Th1 cells. When Th cells were differentiated into Th2 cells, the expression of Socs1 was 23 times higher than that of Th1 cells [26]. In the present study, it was found that the expression of Socs3 was two times...

Fig. 2. LDR- and HDR-induced expression of Th1-Th2-Th3-related genes. Gene expression was downregulated after LDR and upregulated in the HDR group. Gene expression differences were significant with fold changes >2.0; this is an additional criterium of biological relevance. (*P < 0.05 vs control group).

Fig. 3. Effects of LDR and HDR on Th1-Th2-Th3-related cytokine secretion by mouse thymocytes. IFN-γ, IL-2, IL-1β, IL-4, IL-21 and TGF-β concentrations in culture supernatants were determined by ELISA. Mean values of triplicate cultures were obtained for each of three experiments, and results were expressed as relative expression level (%) compared with the control group (*P < 0.05, **P < 0.01).
greater than that in the sham group after 2.0 Gy irradiation, while Socs1 expression was 3.03 times greater, indicating that high-dose irradiation may upregulate Socs3 and induce differentiation of Th2 cells.

IL-1 is a macrophage-derived pro-inflammatory cytokine that assists in T cell activation. The receptor for IL-1 plays an important role in inflammation by activating nuclear factor kappa B and neuro-modulation in the central nervous system [27, 28]. Tbx21 (T-box 21, also known as T-bet/Tbx21) was the other gene showing significant upregulation after 0.1 Gy exposure. This gene encodes a transcription factor that activates IFN-γ production in the Th1 subset and promotes development of Th1 cells while inhibiting Th2 cells [29, 30]. It is also well known that TGF-β1 is a negative immunoregulatory factor secreted by Treg cells. Its main function is to inhibit the proliferation of T cells and the secretion of inflammatory cytokines, and it plays an important role in maintaining immune tolerance and immune balance [31]. In addition, the results of this study indicated that the HDR-upregulated genes were positively regulated cytokines (IL-12b, IL-15, IL-18, GM-CSF, etc.), negatively regulated cytokines (IL-5, TGF-β, IL-10, IL-6, IL-17, IL-23, etc.) and negative regulatory co-stimulatory molecules (CTLA-4). Furthermore, HDR downregulated the early effect of the Gfi-1 and CD124 (IL-4Ra) genes in Th2 cells, so that T cells mainly regulated Th3/Tr1 cell differentiation and induced Th2 cells to inhibit the immune response. At the same time, we found that the cytokines related to Th1-Th2-Th3 cells in the thymus of mice that were most affected by high- and low-dose radiation were TGF-β, C/EBP-β and TNF-α. We further confirmed the expression of Th1-Th2-Th3 cell-related cytokines with ELISA. IFN-γ and IL-2 secretion were positively regulated by LDR irradiation (P < 0.05), which had a negative effect on IL-1, IL-4, TGF-β and IL-21 expression, and there was a significant difference between the LDR and HDR groups, with the HDR group showing an opposite pattern of expression of that of the LDR group.

In summary, LDR and HDR induced different immune effects. LDR induced the Th1 immune response to increase immune function, while HDR induced Th3/Tr1 cells and inhibited the immune response. These experimental results will add a new experimental basis for radiological immunology theory.

CONFLICT OF INTEREST
The authors have declared that there is no conflict of interest.

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