Short Report

Effects of Gamma Irradiation on Proliferation and IL-5 Production of Peripheral Blood Lymphocytes

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ABSTRACT

Gamma irradiation is routinely used for suppression of lymphocyte function in transfusion and transplantation procedures. In recent years, some investigators focused on the effects of ionizing radiation on special aspects of lymphocyte function and considered the possibility of its clinical application for treatment of some immunological disorders. In this study, we evaluated the effects of five different doses of γ-ray on proliferation and IL-5 production of peripheral blood lymphocytes. Lymphocytes were separated from blood and were treated with 5, 10, 20, 30 and 40 Gy irradiation (using a 137 Cs source) and then were cultured for 72 h in the presence of phytohemagglutinin (PHA). The proliferative response of samples was evaluated by MTT assay, and the supernatant of the cells was collected for IL-5 detection. The results showed that the ionizing radiation had a suppressive effect on lymphocyte proliferation. IL-5 production was affected in a dose-response manner, augmented in response to 5 and 10 Gy and reached to its peak value at 20 Gy. At 30 Gy, IL-5 production was diminished lower than peak value, but still remained higher than control baseline, and 40 Gy led to IL-5 values lower than baseline. *Iran. Biomed. J. 8 (4): 211-214, 2004*

Keywords: Gamma irradiation, Lymphocyte proliferation, Th1/Th2 cytokines

INTRODUCTION

The adverse biological effects of ionizing radiation are well known from studies involving atomic bomb survivors, patients undergoing radiotherapy, people exposed to radiation accidents, and different kinds of *in vitro* studies [1]. In irradiated cells, B-lymphocytes show a more pronounced radiosensitivity, but with quicker recovery than T cells [2, 3]. There are some differences among T lymphocyte subpopulations in response to ionizing radiation [4].

Irradiation causes alteration in cytokine release from different cell types [5, 6] and shift in immune responses to Th2 cells [6-8]. Different studies indicate that ionizing radiation causes augmentation of Th2 cytokine production, for example IL-4, IL-5 and IL-10 [9, 10].

However, most studies about the effects of gamma irradiation on cytokine production were carried out in the form of total lymphoid irradiation (TLI) or total body irradiation (TBI). In accordance with *in vivo* studies, it has been shown that gamma irradiation causes a shift to Th2 response in mouse splenocytes. The cells treated with different doses of gamma ray produced less IFN-γ and more IL-5 than the untreated group [10].

In the present investigation, we examined the effects of different doses of gamma irradiation on proliferative response and IL-5 production of peripheral blood mononuclear cells and showed that certain doses of irradiation cause an increase in IL-5 production. This finding may have clinical applications in shifting the immune response to a Th2 type in situations such as autoimmune disease that an exaggerated Th1 type response is destructive, or in certain infectious diseases which

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Th2 response is necessary for eradication of the parasites.

MATERIALS AND METHODS

Lymphocyte preparation. Lymphocyte populations for each test series were obtained from blood sample of a consenting healthy volunteer. The whole blood was applied onto the Ficoll-Hypaque (Biotest, Germany) gradients and centrifuged at 400 ×g for 30 min. Mononuclear cells were isolated and after washing twice, suspended in RPMI 1640 medium (2.5×10^5 cells /ml) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, 50 µg/ml gentamicin, and 2 mM L-glutamine. The suspended cells were placed in sterile tubes for irradiation.

γ- irradiation. For γ-irradiation, sterile tubes containing lymphocytes were placed in a suitable rotator holder in a gamma cell apparatus with 137 Cs source (Isomedix; Nordion, Katana, Ontario, Canada). Five different doses of γ-irradiation from 5 to 40 Gy (5, 10, 20, 30, 40) were used in this study. Subsequently, irradiated and non-irradiated cells were transferred to a 96-well microplate and cultured with 5% CO_2 at 37°C for 72 h (5×10^4/cells 0.2ml in each well). For each dose, six wells (three for stimulation and remaining as baseline) were considered. PHA (1 µg/ml) was used for stimulating lymphocyte proliferation.

MTT assay. Cell proliferation was evaluated by reduction capacity of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to a formazan product [11]. The yellow tetrazolium salt was reduced in metabolically active cells to form insoluble purple formazan crystals, which were solubilized by the addition of a detergent.

MTT was dissolved in PBS at 5 mg/ml. After 72 h, 10 µl of the mentioned concentration of MTT was added to all wells of the microplate and then was incubated at 37°C for 4 h. After this period, the plate was centrifuged at 1,000 ×g for 5 min. Acid-isopropanol (100 µl of 0.04N HCl in isopropanol) was added to all wells and thoroughly mixed to be dissolved. Then, the plate was read on an ELISA reader (Boehring, Elisa Supply Unit, Germany) at 570 nm and a reference wavelength of 650 nm. The % proliferation was calculated by the equation:

%proliferation = [(test cell OD- control cell OD)/ control cell OD] ×100

Cytokine assay. Supernatant of mononuclear cells stimulated with PHA for 72 h was used for measurement of IL-5 production.

IL-5 levels in the supernatants were measured using ELISA method according to the manufacturer's instructions (NIBSC, UK). Briefly, ELISA plates were coated with anti-IL-5 antibody overnight at 4ºC and blocked with 10% FCS-PBS. Samples and dilution rows of purified IL-5 as standards were incubated at room temperature for 2 hours. Biotinylated anti-IL-5 antibody followed by streptavidin-HRP and TMB (3, 3', 5, 5'-tetra-methyl benzidine) substrate was used for detection.

Statistical analysis. All data were analyzed using the Student's paired *t*-test and analysis of variance (ANOVA).

RESULTS

Lymphocyte proliferation. As shown in Figure 1, irradiation significantly decreased the proliferative response of lymphocytes to mitogen. Proliferative response was completely stopped at 20 Gy.

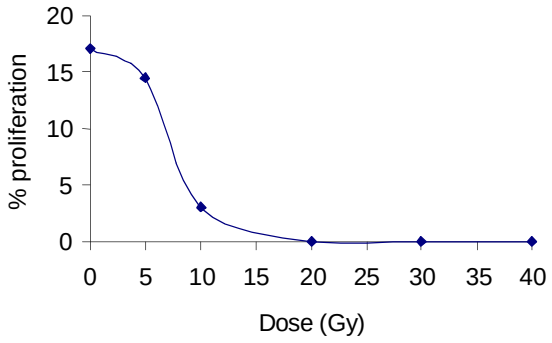


Fig. 1. Effects of different doses of γ-irradiation on peripheral blood lymphocyte proliferation.

IL-5 production. Irradiated lymphocytes which were treated with PHA, progressively increased the secretion of IL-5 after 72 h when compared to the stimulated controls. As seen in Figure 2**,** this effect was started from 5 Gy and reached to its peak values at 20 Gy. IL-5 production significantly augmented at 10, 20 and 30 Gy ($P<0.0001$). At 40 Gy**,** IL-5 concentration decreased to values less than control baseline.

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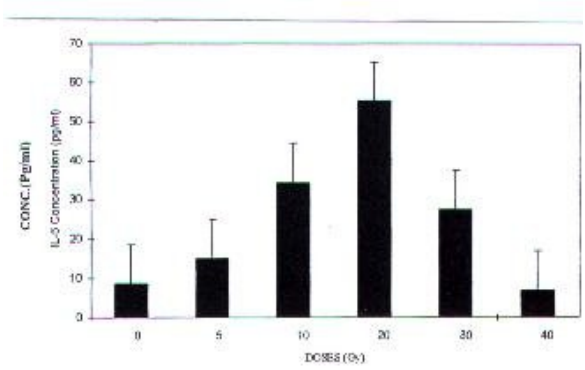


Fig. 2. Effect of different doses of γ-irradiation on IL-5 production from peripheral blood lymphocytes.

DISCUSSION

The variations in radiosensitivity of cells involved in immune reactions are well known, but little is known about the regulation of cytokine release by irradiated cells [3, 12]. The previous animal studies using TLI-treated mice and or *in vitro* irradiated lymphocytes indicate some differences in cytokine releasing among various cell types. Our results show that sub lethal doses of irradiation had an inhibitory effect on proliferation of lymphocytes. There are a few investigations regarding the effects of irradiation on cytokine production by different subpopulations of lymphocytes. Bass *et al.* [13] demonstrated that spleen cells of TLI-treated mice secrete IL-5 at the same level as normal untreated cells and 59-88% of the mean normal level of IL-4, 5-9% of the mean normal level of IL-2 and 6-20% of the mean normal level of IFN-γ. Galdiero *et al.* [3] applied sub lethal doses of γ-ray and showed the increased production of IL-4 after lymphocytes stimulation following irradiation. In contrast, Field and Rouse [14] reported that repeated *in vivo* antigen priming of TLI-treated mice results in secondary cytokine responses characterized by increased production of IL-4 and decreased production of IFN-γ. They also showed a direct association between increased productions of Th2 versus Th1 cytokines and diminished anti-donor CTL activity in transplantation after TLI [6]. Other investigations show that T cells from TLI-treated mice produce more IL-4 and less IL-2 and IFN-γ than normal counterparts [14, 15]. A recent *in vitro* study carried out by Han *et al.* [10] using mouse splenocytes shows that γ-irradiation reduces IFN-γ mRNA expression and leads to IL-5 mRNA and transcription factor GATA-3 induction. GATA-3, which is a transcription factor and selectively expressed on naive and Th2 cells, inhibits Th1 development and modulates IL-4 and IL-5 production [16].

Our results provide direct evidence regarding similar *in vitro* effect of γ-irradiation on IL-5 production by human peripheral blood lymphocytes.

TH1 cells produce IFN-γ and IL-2, which are important mediators for the development of organ-specific autoimmune disorders [17]. Since the positive influences of Th2 type response in autoimmune disorders, specially in organ specific types, are well known [18, 19], Th2 driving effect of γ- irradiation may have beneficial effects in the treatment of autoimmune diseases. Furthermore, IL-5 as a Th2-cytokine has an important role in stimulation, proliferation and differentiation of B cells and eosinophils and is a critical factor in defense against parasitic diseases [20]. Thus, irradiation may have a beneficial effect on anti parasitic immune response.

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