Original Article

The Possible Protective Effect of Interferon-α and Bone Marrow Transplantation on Lymphoid Tissues in Irradiated Rats

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Abstract

The current work was done to investigate whether interferon-α (IFN-α) and/or bone marrow (BM) transplantation have role in reducing the dangerous effect of γ-irradiation on lymphoid tissues. Control group, BM injected group, IFN-α treated group, irradiated group, irradiated BM injected group, irradiated IFN-α treated group and irradiated BM and IFN-α treated group were used. All animals groups were sacrificed after 5 weeks of final treatments. Cytokines assay of serum IL-2 and TNF-α concentrations were determined in serum. Histopathological observations and connective-tissue fibrillae were detected in spleen, lymph node and thymus tissues. Exposure to γ-irradiation recorded a significant decrease in serum IL-2, TNF-α values, decreased in cell populations and increase of collagen deposition in spleen tissue, reduction in size and shrinkage appearance of lymph node tissue and great thickness in trabeculae and heavy cellular medulla in the thymus tissue. BM transplantation represented a significant increase in IL-2, no change in TNF-α, increase in cellularity of periarteriolar lymphocyte sheath in spleen tissue and the presence of follicular hyperplasia in lymph node tissue. After IFN-α injection, a significant increase in IL-2, no change in TNF-α and normal appearance in spleen or lymph node tissues was detected. γ-radiation exposure followed by BM cells transplantation exerted a non significant change in IL-2 and a significant elevation in TNF-α when compared to the irradiated group, resulted in decreased cellularity and great deposition of collagen fibres in spleen tissue and increase in Hassell's corpuscles in the medullary region of thymus tissue. Return to normal appearance of spleen, lymph node and thymus tissues was noted when irradiated rats treated with IFN-α either alone or combined with BM cells transplantation. Also a normalize IL-2 and TNF-α to reach control values when dual treatments of irradiated rats by BM cells transplantation and IFN-α were done. In conclusions, it’s important for the usage of IFN-α treatment during BM transplantation in irradiated animals.

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Introduction

Ionizing radiation causes a number of various cytological, histological and physiological injuries (Hall and Angele, 1999; Cornforth, 1998; Ward, 1998). Also the use of whole-body irradiation destroy lymphocytes in lymphatic tissue and to treat lymphocytic neoplasia exploits the radiosensitivity of these cells, but its application is limited by the damage to other radiosensitive tissues, especially the bone marrow (Birch et al., 1986). Besides, the radiation may cause bone marrow suppression and depletion of peripheral blood lymphocytes, and lead to severely inhibit the function of immune system such as making the exposed animals susceptible to opportunistic pathogens, more easily to be infected, and sometime to be lethal (Monje and Palmer, 2003; Dillman, 2006). Several studies reported that immunostimulators such as interferon’s and interleukins have been reported to render radio protective effect on the mice (Xiaogang et al., 2010)
The IFNs are a family of natural glycoproteins and regulatory cytokines with pleiotropic cellular functions, such as anti-viral, anti-proliferative and immunomodulatory activities (Baron et al., 1994; Hertzog et al., 1994). They were first discovered as anti-viral soluble protein (Camps et al., 1993; Schvarcz et al., 1991), consist of type I interferon, which includes interferon α, β and ω, and type II interferon, which is interferon γ. On the other hand, IFNs also has significant antitumor activity through the inhibition of angiogenesis in experimentally-induced tumors in animals (Wada et al., 2007). IFN- α was the main cytokine induced in the innate immune response directed against viral infection (Fensterl and Sen, 2009). IFN-α has immunomodulatory properties (Nakamura et al., 2007; Yamamoto et al., 2004) and have at least 14 subtypes (Pestka and Meager, 1997; Pestka, 1997). IFN- α is rapidly induced with a high expression level and secreted into the blood circulation in response to viral infection in many types of cells, and then binds to a specific cell surface receptor and triggers intracellular reactions that lead to the transcriptional induction of IFN-stimulated genes (ISGs) (Kovarik et al., 2007). Marschall et al. (2003) reported that the therapeutic effects of IFN-α on tumor cells were based on Sp1- and/or Sp3-mediated inhibition of VEGF transcription both in vivo and in vitro. (Wada et al., 2007).

Autologous or syngeneic and allogeneic bone marrow transplantation (BMT) is increasingly used in the therapy of lymphohematopoietic and solid malignancies, as well as in non-malignant disorders such as thalassemia and immunodeficiency (OReilly, 1983; Lucarelli et al., 1985; Champlin and Gale 1987; Vossen, 1987). Also the preclinical and clinical studies have demonstrated that bone marrow stromal cells (MSCs) can be used for tissue repair (Yoon et al., 2005).

Our work was designed to evaluate the possible protective effect of interferon-α and/or BM transplantation on lymphoid tissues in irradiated rat

Materials and Methods

Mature male albino rats of pure strain (Rattus albinus) ranging from 110-150 body weight were obtained from the animal house of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. The animals were maintained on a commercial standard pellet diet and tap water ad libitum.

Radiation facility

Whole body γ- irradiation was performed with a Canadian 137Cs Gamma Cell-40 at the National Centre for Radiation Research and Technology, Cairo, Egypt; at a dose rate of 1.2 R/second. Rats were exposed to 5 Gy whole body γ- radiations delivered as an acute single dose of 5Gy.

Interferon alpha (IFN-α)

Interferon alpha is prepared from culture of genetically modified Escherichia coli using recombinant DNA technology. Each vial of interferon alpha contains 3 million IU. Rats were injected subcutaneously with 10 units of interferon alpha once a week for 5 weeks (Muriel and Castro, 1997).

Bone Marrow (BM) transplantation

Donors and recipients rats were chosen of the same inbred strain, brother to brother (isologues or synergetic or allogeneic transplantation). Femur bones were dissected out and cleaned. The ends of the bones were chipped by a bone nipping forceps. Then the marrow was blown out of the femur into isotonic solution under sterilized conditions inside a laminar flow cabinet. The marrow was collected into a sterile container surrounded by ice cubes, and mixed by drawing and expelling it several times from the syringe without needle in order to avoid mechanical damage to the cells. Total viable cells of about 75 ×10⁶ ± 5% were injected intravenously (IV) through the caudal vein.

Groups of animals under investigation:

Healthy Swiss albino rats were divided into 7 groups of 6 animals in each. G1: Vehicle control animals. G2: Group of animals IV injected with BM cells (75 × 10⁶ cells) through the caudal vein. G3: Group of animals SC injected with 10 units/week x 5 of IFN-α. G4: Group of animals exposed to 5Gy of whole body γ-radiation. G5: Group of animals exposed to 5Gy of γ-radiation and IV injected with BM cells three hours after γ-radiation exposure (Decleve et al., 1972). G6: Group of animals exposed to 5Gy γ-radiation and SC injected with IFN-α. G7: Group of animals exposed to 5Gy γ-radiation, treated with BM cells and SC injected with IFN-α. After 5 weeks of final treatments all groups of animals were sacrificed. Blood was collected from ether anesthetized animals by cardiac puncture and centrifuged at 3000 rpm for 15 minutes for Serum preparation. Serum was stored frozen at -20°C until used. Parts of the spleen, lymph nod and thymus tissues were excised, fixed in 10% formalin for 48 hours, then dehydrated, processed and embedded in paraffin blocks. For cytokines assay serum IL-2 and TNF-α concentrations were determined by using ELIZA according to Chan and Perlstein (1987) and Aramachi, (1989) respectively. For histopathological observations, sections of (5-6 μm) thickness from spleen, lymph nod and thymus tissues were stained with haematoxylin and eosin (H&E) according to Drury and Wallington, (1976). For connective-tissue fibrillae and reticulum detection Mallory’s stain was used according to Mallory, (1900).
Results

1-Cytokines assays:

Results in Fig. (●) recorded that treatment of rats by BM cells transplantation or IFN-α represented a significant increase in IL-2 (p<0.05) as compared to control group while TNF-α represented no change. A significant decrease was obtained in serum IL-2 and TNF-α values (p<0.05) post 5 Gy of γ-irradiation. BM cells transplantation following γ-irradiation exposure exerted no significant change in IL-2 (p<0.05) while, TNF-α (p<0.05) had a significant elevation when compared to the irradiated group. IFN-α post irradiation showed a significant increase change in IL-2 (p<0.05) while, TNF-α (p<0.05) had a significant decrease when compared to the irradiated group (p<0.05). Dual treatments of irradiated rats by BM cells transplantation and IFN-α normalize IL-2 (p<0.05) and TNF-α (p<0.05) to reach control values.

Fig. (●): Effect of BM cells transplantation and IFN-α on IL-2 and TNF-α in irradiated rats.

Values expressed as mean±SE, ANOVA test.
*: Significance from control group. #: Significance from irradiated group.

2-Histopathological observations:

H and E stain

1-Spleen tissue

The spleen is the largest secondary immune organ in the body and is responsible for initiating immune reactions to blood-borne antigens and for filtering the blood of foreign material and old or damaged red blood cells. These functions are carried out by the two main compartments of the spleen, the white pulp (including the marginal zone) and the red pulp, which are vastly different in their architecture, vascular organization, and cellular composition (Fig. A 1, 2). Increase in cellularity of marginal zone of periarteriolar lymphocyte sheath of white pulp after treatments with BM transplantation or IFN-α were showed (Fig. A 3, 4).

When experimental animals exposed to γ-irradiation the spleen tissue represents decreased in erythroid and myeloid cell populations in the red pulp and replaced by stromal cells (Fig. B9). In the white pulp increased the sickness of the central arteriole and the presence of many apoptotic cells were observed (Fig. B 10). The SC injection of the irradiated animals by IFN-α recorded recurrence of normal appearance of spleen tissue. On the other hand treatment of the irradiated animals by IV injection with BM cells three hours after γ-irradiation exposure, resulted in decreased cellularity of both the white pulp and red pulp in addition to the presence of thickened congested central arteriole which full of accumulated blood cells (Fig.B 11). Recurrences of normal appearance of follicle, marginal zone and the periarteriolar lymphocyte sheath were noted when animals exposed to 5Gy γ-ray radiation either treated with SC injected with IFN-α (Fig. B 12) or treated with BM cells and SC injected with IFN-α (Fig. B 13, 14).

2-Lymph node tissue

The lymph nodes are organized lymphoid organs that contain lymphocytes within a fine reticular stroma. The structures within a lymph node include the capsule, subcapsular sinus, cortex (B cell zone with follicles and germinal centers), paracortex (T cell zone), medullary sinuses, medullary cords and hilus. Control lymph node recorded the presence of capsule, cortex and medulla (Fig. C 1) The medullary sinuses are composed primarily of lymphocytes, reticular fibres providing the support framework, reticular cells (fibroblast-like cells that secrete the reticulin) and macrophages (Fig. C 2). Treatment of the experimental animals with BM transplantation recorded as decreased paracortical area and cellularity, increased medullary area with decreased medullary cellularity and the presence of follicular hyperplasia (Fig. C 3, 4). Mean while the treatment of the experimental animals with IFN-α recorded the normal appearance and presence of germinal centres (Fig. C 5, 6).

Great reduction in lymph node size showing shrinkage appearance after exposure to γ-irradiation (Fig. D 7). Also loss of lymphocytes in conjunction with eosinophilic cellular debris and dark pyknotic nuclear debris in the medullary region with the presence of thickness of the reticular fibres in Fig. (D 8). Treatment of the irradiated animals by BM transplantation or IFN-α injection recorded normal appearance of lymph node tissue with the presence of lymphoid necrosis within the cortex and regions of the paracortex (Fig. D 9, 10, 11 and 12). On the other hand the structure of lymph node tissues from rats exposed to γ-irradiation , BM cells and IFN-α represented the reappearance.

3-Thymus tissue

The thymus is a primary or central lymphoid organ in which T lymphocytes undergo differentiation and maturation...
Autonomously within the cortex, without the need for antigenic stimulation, and it is essential for the normal development and function of the immune system. The control thymus composed of two compartments cortex and medulla (Fig. E1). In medullary region Hassall's corpuscles and T lymphocytes were observed (Fig. E2). Treatment of the experimental animals by BM transplantation (Fig. E3) or INF-α (Fig. E4) recorded anon significant change in thymus tissue structure.

Exposure of rats to γ- radiation showed thickened cortex (Fig. F5), great thickness in trabeculae and heavy cellular medulla (Fig. F6). Some degenerative changed areas and increased Hassell's corpuscles were also predicted in the thymus tissue (Fig. F5). Treatment of irradiated rats by BM represented increase in Hassell's corpuscles in the medullary region (Fig. F7). On the other hand the Treatment of irradiated rats by INF-α showed increase in Medulla cells form a layer around Hassell's corpuscles and aggregation of macrophages in the cortex region in (Fig. F 8 and 9).

However the treatment of irradiated rat by BM cells and IFN-α recorded normal appearance of thymus tissue in medullary region and cortex (Fig. F 10, 11 and 12).

3-Histochemical observations

Mallory’s stain

1- Spleen tissue

In spleen tissue Mallory’s stain showed normal appearance of control sections with thin collagenous bundles encircle the capsule and distributed throughout the red and the white pulp in addition to the presence of blue staining connective tissue (Fig. G1). Treatment of the experimental animals by IFN-α represented decrease in collagenous bundles distribution throughout the red and white pulp. Muscular red staining substances encircled the white pulp permanently observed in the white pulp (Fig. G2). On the other hand treatment of the experimental animals by BM transplantation spleen section
Fig. B. Photomicrographs of sections in spleen tissue of male Wistar rats stained with H&E. Treatment of the experimental animals by γ-irradiation showing, replacement of the erythroid and myeloid cells by stromal cells (↑), fibrotic deposition around the central arteriole (blocked arrow) and the presence of many apoptotic cells (▲) in 9(x100) and 10 (x400). Treatment of experimental animals by γ-irradiation followed by IV BMs injection showed decreased in cellularity of both the white pulp (↕) and red pulp and great of fibres deposition around the congested central arteriole (↓) in 11 (x100). Treatment of experimental animals by γ-irradiation followed by IFN-α injection showed normal appearance of white pulp (↓) and red pulp (↑) in 12 (x100). Treatment of the experimental animals with γ-irradiation, BM cells and IFN-α showed recurrences of normal appearance of follicle (↓) marginal zone (curved arrow) and the periarteriolar lymphocyte sheath in 13 (x100) and red pulp cellularity (↕) in 14 (x400).

illustrated some increased and thickened collagenous bundles intersecting the red and white pulp (Fig. G3). However when rats exposed to γ-radiation, great disappearance of distributed collagenous bundle throughout the red and the white pulp was shown. Also disappearance of blue staining connective tissue which replaced by a cytoplasmic red component in red
Fig. C. Photomicrographs of sections in lymph nod tissue of male Wistar rats stained with H&E. Control lymph node represented capsule (curved arrow), cortex (↓) and medulla (↕) x100 in 1 (x100). The medullary sinuses are composed primarily of lymphocytes (↑) reticular fibres (▲), reticular cells and macrophages in 2 (x400). Treatment of the experimental animals with BM transplantation recorded the presence of follicular hyperplasia (curved arrow) in 3 (x100) and 4 (x400). Treatment of the experimental animals with IFN-α recorded the normal appearance and presence of germinal centres (↓) in 5 (x100) and 6 (x400).

- Lymph nodes tissue:
  - Mallory’s stain showed normal appearance of control lymph nod sections with thin collagenous bundles encircled by the capsule and distributed throughout the cortex and the medulla (Fig. H8). IFN-α treatment section illustrated decreased collagenous connective tissue bundles throughout the cortex and the medulla (Fig. H9). Great disappearance of collagenous connective tissue bundles throughout the cortex and the medulla were done when experimental rat treated by BM transplantation (Fig. H10). Shrinkage lymph appearance of irradiated rats showed increased and thickened collagenous bundles intersecting the cortex and the medulla with thickened septa were noticed in Fig. (H11). The same observations were illustrated when irradiated rats treated by BM transplantation (Fig. H12). However treatment of irradiated rats by IFN-α recorded some recurrence of collagenous connective tissue in lymph node tissue (Fig. H13). Normal appearance of collagenous bundles distribution throughout the cortex and medulla in addition to the presence of blue staining connective tissue were showed in lymph node tissue when irradiated rats treated by BM transplantation and IFN-α (Fig. H14).

- Thymus Tissue
  - Mallory’s stain showed normal appearance of control sections with thin collagenous bundles encircle the capsule and distributed throughout the cortex and the medulla of thymus tissue section (Fig. I 1).
Fig. D. Photomicrographs of sections in lymph nod tissue of male Wistar rats stained with H&E. treatment of the experimental animals by $\gamma$-irradiation recorded great reduction in lymph node size showing shrinkage appearance In 7 and in 8 the loss of lymphocytes, dark pyknotic nuclear debris in the medullary region (curved arrow) and the presence of thickness of the reticular fibres (↓). Lymph nod tissue represented lymphoid necrosis within the cortex and regions of the paracortex (↑) when the irradiated animals treated by BM in 9 and10 (x100 and 400 respectively) or IFN-α in 11 and 12(x100 and 400 respectively). Notice the reappearance of lymphoid follicle and paracortical zone (↓) and medullar zone (↕) in 13 (x100) after treatment of irradiated rat by BM cells and IFN-α.

Treatment of the experimental animals by IFN-α represented some increase of the previous structure (Fig. I 2). Treatment of experimental animals by BM transplantation or $\gamma$- radiation exposure illustrated clearly great reduction in collagenous bundles distributed throughout the cortex and the medulla of thymus tissue section. Small collagenous bodies enircle the capsule and low aeries of medullary region (Fig. I 3, 4). Some reappearance of collagenous bodies in thymus medullary region was showed when irradiated rats treated by BM transplantation (Fig. I 5) or IFN- α (Fig. I 6). Normal appearance of collagenous bundles distribution throughout the cortex and medulla in addition to the presence of blue staining connective tissue were showed in thymus tissue.
Discussion

The effects of ionizing radiation on organs tissues have been studied in detail for many years (Cornforth, 1998; Fedoročko et al., 1996; Ploskonosova et al., 1999). Also Le (2010) reported that the exposure to radiation has been shown to induce the formation of senescence markers could persist long-term in vivo, possibly contributing to the permanent reduction in tissue functionality intact to immune system. The immunosuppression that was caused by γ-irradiation was due to the differential regulation of the T-helper cell type 1 and T-helper cell type 2 cytokine gene expressions (Seon-Kyu et al., 2002).

IL-2 is a cytokine released by T helper lymphocytes. It promotes the generation, proliferation, and differentiation of T lymphocytes, enhances the activity of natural killer cells; induces the generation of lymphokine-activated killer cells and promotes the production of antibodies by B lymphocytes. Through these mechanisms, it plays an important role in antitumor immune responses (Gong, 2003). In agreement with literature, the present study demonstrated decreased IL-2 level in irradiated animals. Bass et al. (1989) demonstrated that spleen cells of total lymphoid irradiated (TLI) mice, secrete 5-9% of the mean normal level of IL-2. Other investigations show that T cells from TLI-treated mice produce more IL-4 and less IL-2 and IFN-γ than normal counterparts (Adkins et al., 1993 and Field et al., 1997). γ-irradiation is known to significantly inhibit the proliferation of effective T cells by reducing the levels of Th1 type cytokines (such as IL-2) (Han et al., 2005).

Tumor necrosis factor alpha (TNF-α) is a 17 kilodalton cytokine that is synthesized by monocytes/macrophages, natural killer cells/large granular lymphocytes, and T lymphocytes subsets. The present results showed that 5Gy γ-irradiation induced a significant decrease in TNF-α, which is in agreement with Tsukimoto et al.

Fig. E. Photomicrographs of sections in thymus tissue of male Wistar rats stained with H&E. Control thymus showed cortex (↑) and medulla (↕) in 1(x100). In medullary region, Hassall's corpuscles (↕) and T lymphocytes (▲) were observed in 2 (x400). Treatment of experimental animal by BM or INF-α represent normal structure of thymus tissue in 3 and 4 respectively (x100). Star represents high magnification (x400) when irradiated rats treated by BM transplantation and IFN-α (Fig. I 7).
Fig. F. Photomicrographs of sections in lymph node tissue of male Wistar rats stained with H&E. Treatment of the experimental animals by γ-irradiation, the thymus tissue showed thickened cortex and heavy cellular medulla, some degenerative changed areas and increased Hassell's corpuscles (curved arrow) in 5 and great thickness in trabeculae (↓) in 6 (x400). Treatment of irradiated rats by BM represented increase in Hassell's corpuscles (†) in the medullary region in 7 (x400). Treatment of irradiated rats by INF-α showed increase in Medulla cells form a layer around Hassell's corpuscles (▲) in 8 (x400) and aggregation of macrophages (↓) in the cortex region in 9 (x400). Treatment of irradiated rat by BM cells and IFN-α recorded normal appearance of thymus tissue in 10 (x100), medullary region (↕) in 11(x400) and cortex (↓) in 12 (x400).

(2009) who proved the anti-inflammatory effects of γ-irradiation followed by suppression of TNF-α production, in cells of mouse macrophages cell line. TNF induced by irradiation could regulate the BM cells apoptosis in vitro and in vivo which is a crucial event in BM dysfunction (Cachaço et al., 2010). The ability to identify histopathological changes in lymphoid tissues was highly dependent on the severity of the specific lesion and the tissue compartment measured (Germolec et al., 2004). Overall, histopathological changes...
Fig. G. Photomicrographs of sections in spleen tissue of male Wistar rats stained with Mallory’s stain showing: (1) normal control, (2) IFN-α treatment, (3) BM transplantation treatment, (4) γ-irradiation treatment, (5) γ-irradiation and BM transplantation treatments, (6) γ-irradiation and IFN-α treatments, (7) γ-irradiation, BM transplantation and IFN-α treatments (x100)
Fig. H. Photomicrographs of sections in lymph nod tissue of male Wistar rats stained with Mallory’s stain showing: (8) normal control, (9) IFN-α treatment, (10) BM transplantation treatment, (11) γ-irradiation treatment, (12) γ-irradiation and BM transplantation treatments, (13) γ-irradiation and IFN-α treatments, (14) γ-irradiation, BM transplantation and IFN-α treatments (x100), (star x400) and (● x40)
Fig. 1. Photomicrographs of sections in thymus tissue of male Wistar rats stained with Mallory’s stain showing: (1) normal control, (2) IFN-α treatment, (3) BM transplantation treatment, (4) γ-irradiation treatment, (5) γ-irradiation and BM transplantation treatments, (6) γ-irradiation and IFN-α treatments, (7) γ-irradiation, BM transplantation and IFN-α treatments (x100).
were most frequently and most consistently reported in the thymus cortex and medulla, and in the spleen and lymph node follicles (cellularity and germinal centre development) (Germolec et al., 2004).

Since (1966) Congdon, reported that the exposure to whole-body radiation reducing the size of normal lymph nodes in mice. In our study exposure to γ- radiation showed that spleen tissue represents decreased in cell populations in the red pulp replaced, by stromal cells. In addition great disappearance of distributed collagenous bundle and the presence of increased the sickness of collagen deposition around the central arteriole. Also the thymus tissue represented thickened cortex and heavy cellular medulla, some degenerative changed areas and increased Hassell's corpuscles and great thickness in trabeculae. Those may be due to clean up the damaged thymocytes, and other possibility, as production of cytokines which may contribute to the rapid proliferation (Fukumoto et al., 1999). The structural changes were observed in spleen after whole body irradiation may be due to mature lymphocytes which showed early cytonecrosis, characterized by marked cellular shrinkage without fragmentation and usually were phagocytized whole by reticular macrophages, subsequently fragmented and were digested (Jordan, 1969).

The great reduction size and shrinkage appearance in lymph node tissue in addition to the presence of thickness of the reticular fibres context with the findings of Thomas Fisher (2008) and Matthias et al., (2009) due to the production of free radicals in the cell (Sung Jae, 2003). Al-Bayati (2002) reported that the changes in all lymph node structures, including the stroma; in the hyperplasia stage, and then in the atrophy and shrink of all lymph nodes may be due to the loss of corticosteroids. These corticosteroids shrink the immune system along with the thymus, lymph nodes and all the peripheral tissues.

The bone marrow serves as a reservoir for different classes of stem cells. In addition to haemopoietic stem cells, the bone marrow comprises a population of marrow stromal cells or mesenchymal stem cells (MSCs) (Mohamad et al., 2007). In the present study, BM transplantation to irradiated animals induced a slight reduction in IL-2 concentration compared to irradiated animals. Wang et al. (2002) explained that IL-2 concentrations in recipient mouse serum were relatively low, because of cytokine autocrine and paracrine physiological characteristics, their expression in a microenvironment may be sufficient to reconstitute the immunological and hematopoietic depression after BM transplantation. Also, BM transplantation could cause the lack of IL-2 producing cells and/or the increased activity of suppressor cells of the helper function. Also, serum level of soluble IL-2 receptor was increased in patients in whom acute graft-versus-host disease developed after allogeneic bone marrow transplantation and its level was significantly related to disease severity (Nakamura et al., 2004). The depression in IL-2 level in the present results support the successful engraftment of bone marrow cells.

Serum TNF may be produced as a result of latent infections or immunological reaction against non-HLA allogeneic antigens. Antin and Ferrara, (1992) demonstrated TNF-α release from endothelial cells and keratinocytes in response to injury. The observed in serum TNF levels by irradiation and BMT amelioration compared to the irradiated group may be related to absence of the immunological reaction against non-HLA allogeneic antigens. This is supported by the experimental observation that TNF is produced in allogeneic mixed lymphocyte reaction (Shalaby et al. 1988). Furthermore, neutralization of TNF-α have been reported by Brownand Thiele, (2000) to reduce complications after BMT.

The results of our transwell experiments showed that the BM transplantation represent increase in cellularity of marginal zone of periarteriolar lymphocyte sheath of white pulp in spleen tissue and increased medullary area with decreased medullary cellularity and the presence of follicular hyperplasia in lymph node tissue. However thymus tissue recorded anon significant change in its tissue structure. Also the lymphoid tissues represented some what increased in collagenous bundles deposition. The increase in cellularity may be an expression of a focal expression of a focal recovery of the host immune mechanism with destruction of the foreign bone marrow graft in that particular region of the spleen or lymph node (Congdon, 1966).

Regarding the effect of IFN-α on lymphoid tissue, increase in cellularity of marginal zone of periarteriolar lymphocyte sheath of white pulp were observed in spleen tissue and normal appearance and presence of germinal centres in lymph node tissue. In addition some decrease in collagenous bundles distribution was detected. IFN-α has been shown to possess a variety of immuno modulatory effects, including antitumor effects both in animal tumor models (Brunda et al., 1984). Hein and Supersaxot (1988) showed that IFN causes retention of normally recirculating lymphocytes in lymph nodes is consistent with effects observed in other species. Also, IFN causes enlargement and increased cellularity of lymph nodes and lymphopoenia when injected into rodents (Gresser et al., 1981; Schattner et al., 1983). Injection with BM cells three hours after γ-radiation exposure resulted in decreased cellularity of both the white pulp and red pulp in addition to the presence of great deposition of collagen fibres around the congested central arteriole increase in the thickness of central arteriole which full of accumulated blood cells in spleen tissue. These may be due to radiation exposure which exhibit impaired endothelial
responses in regions that are more prone to the detrimental effects of disturbed flow and thus have increased atherosclerosis. The endothelial damage at a site predisposed to atherosclerosis in association with the disturbed flow characteristic of that site could account for the exacerbated atherosclerosis in BMT rat (Natalie et al., 2001) represented increase in Hassell’s corpuscles in the medullary region. The normal appearance of lymph nod tissue context with the findings of Van Bekkum, (2003) who reported that allogeneic BMT after total body irradiation was found to cure hereditary as well as inducible forms of AID. While the presence of lymphoid necrosis within the cortex and regions of the paracortex of lymph nod tissue may be responsible for cytorecrosis of radiation exposure (Jordan, 1969).

In accordance with the present results, Crossley et al. (1996) demonstrate that serum level of IL-2 increases after administration of IFN-α due to increased natural killer cell activity with consistent increase in T helper1 activity and a decrease in autoantibody production, which may be related to the beneficial effects of this cytokine. The present results showed that administration of IFN-α after γ-irradiation emphasized the decreased serum level of TNF-α followed the exposure to radiation to come back to normal value. This is in agreement with Nestel et al. (1992) and Hill et al. (1997) who mentioned that the production of TNF-α by monocytes and macrophages is transuded by 2 signals. The first is a priming signal that may be provided by interferon (IFN)-γ and radiation. The second is a triggering signal provided by bacterial products such as LPS. Kosar et al. (1999) showed also that TNF-alpha was increased post-treatment with INF-α indicating presence of immune system activation and endothelial injury/activation. TNF-alpha might be potentially applied as a therapeutic agent in order to enhance sensitivity to γ-irradiation exposure in radiotherapy (Youn Yi et al., 2010).

The return to normal appearance and normal appearance of collagenous bundles distribution throughout the lymphoid tissues when experimental animals exposed to γ-radiation either treated with BM cells and SC injected with IFN-α represent the synergistic effect of BM and IFN-α. The immunosuppressive effect of γ-irradiation is well known (Seon-Kyu et al., 2002). IFN-α has also been shown to possess a variety of immuno modulatory effects (Brunda et al., 1984) and enhances biological defence activities against oxidative stress (Lu et al., 2002). Also Hoffmann et al., (1994) accepted from experimental strategies the future treatment of squamous cell carcinomas by IFN-α, and radiation exposure.

In Conclusions, it’s important of IFN-α treatment usage during BM transplantation and radiotherapy treatment.

References


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