RADIATION-INDUCED CHANGE IN LYMPHOCYTE PROLIFERATION AND ITS NEUROENDOCRINE REGULATION: DOSE-RESPONSE RELATIONSHIP AND PATHOPHYSIOLOGICAL IMPLICATIONS

Shu-Zheng Liu □ MH Radiobiology Research Unit, Jilin University Health Sciences Center, Changchun 130021, People's Republic of China

□ *Cellular activities are regulated by intracellular signals initiated by stimulation from the external and internal environments. Different signal pathways are involved in the initiation of different cellular functions. In connection with cell proliferation in response to mitogenic stimulation, the dose-effect relationship of the magnitude of ^3H -TdR incorporation into lymphocytes after exposure to different concentrations of concanavalin A (Con A) showed an inverted U-shaped curve in the concentration range 2–30 $\mu\text{g}/\text{ml}$. In previous studies it has been observed that the stimulatory effect of Con A (5 $\mu\text{g}/\text{ml}$) on lymphocyte proliferation was potentiated by whole-body irradiation (WBI) with low dose (0.075 Gy) and suppressed by WBI with high dose (2 Gy). When different concentrations of corticosterone, ranging from 10^{-12} to 10^{-7} M, were added to the Con A-stimulated lymphocytes, low-concentration stimulation and high-concentration suppression of lymphocyte proliferation were demonstrated. In the presence of 5×10^{-12} M (subphysiological concentration) of corticosterone the proliferation of thymocytes and splenic T cells in response to Con A was further up-regulated after low-dose radiation. Low-dose radiation (0.075 Gy) caused lowering of serum ACTH and corticosterone concentration as well as down-regulated transcription of the hypothalamic proopiomelanocortin gene. The present paper intends to show that multiple neurohormonal factors, including the pineal gland and neurotransmitters, in addition to the hypothalamic-pituitary-adrenocortical axis, are involved in the stimulation of immune responses induced by low-dose ionizing radiation. The complex nature of the interrelationship between the intracellular signaling of lymphocytes and the neuroendocrine regulation after WBI is discussed.*

Keywords. lymphocyte proliferation, nonlinear dose-response curve, signal molecules, neuroendocrine regulation, pineal gland, catecholamines

INTRODUCTION

Cell proliferation is a basic process constantly going on in the body in response to changes in the external and internal environments. In the immune system, lymphocyte proliferation is often taken as an important parameter indicating the status of the body's defenses. The stimulatory effect of whole-body X-irradiation with a low dose (0.075 Gy) and suppressive effect of a high dose (2 Gy) on concanavalin A (Con A)-induced lymphocyte proliferation have been repeatedly demonstrated (Troup and Anderson, 1982; James and Makinodan, 1988; Makinodan and James, 1990; Liu *et al.*, 1993;

This work was supported by grants from NSFC.

Address correspondence to Shu-Zheng Liu, MH Radiobiology Research Unit, Jilin University Health Sciences Center, Changchun 130021, People's Republic of China. E-mail: drliusz@yahoo.com

Liu, 1998). In a previous paper the dose–effect relationship of immunologic changes following whole-body X-irradiation and some of the mechanisms of low-dose radiation-induced lymphocyte stimulation were analyzed (Liu, 2003). It was demonstrated that intercellular reactions between the antigen presenting cells and lymphocytes and the facilitation of the intracellular signaling cascades thus initiated in the lymphocytes were important molecular mechanisms of T-cell activation induced by low-dose radiation. Among the signal pathways involved, up-regulation of calcium/PKC signals occupy a pivotal position in low-dose radiation-induced potentiation of lymphocyte proliferation. However, in the intact organism, cellular processes are influenced by systemic regulation, especially that from the neuroendocrine system (Besedovsky and Sorkin, 1979; Besedovsky and Rey, 1996; Haddad *et al.*, 2002). The present paper may be considered a continuation of the mechanistic analysis of the augmentation of lymphocyte proliferation in response to low-dose radiation. The involvement of neuroendocrine factors in the signal transduction pathways of lymphocyte activation and proliferation induced by low-dose radiation is reviewed, with emphasis on the possible role of corticosteroids, melatonin, and catecholamines.

EFFECT OF CORTICOSTEROIDS ON CELL PROLIFERATION

Cellular activities are regulated by intracellular signals initiated by stimulation from external and internal environment. Different signal pathways are involved in the initiation of different cellular functions. Lymphocyte proliferation response to Con A is often used as a measure because this is the mitogen most studied in the research of activation and proliferation of murine T lymphocytes (Leon, 1971; Alimkhodzhaeva *et al.*, 2002; Goytia-Acevedo *et al.*, 2003). The proliferative response of thymic and splenic T lymphocytes to Con A was measured by ^3H -TdR incorporation. The dose–effect relationship of the magnitude of DNA replication represented by incorporation of ^3H -TdR into lymphocytes after exposure to different concentrations of Con A ranging from 2 to 30 $\mu\text{g}/\text{ml}$ showed an inverted U-shaped curve (Figure 1, unpublished data).

The left panel of Figure 1 shows the dose–response relationship of the proliferation of mouse thymocytes to 2–30 $\mu\text{g}/\text{ml}$ of Con A, demonstrating a peak proliferation at a concentration of 15 $\mu\text{g}/\text{ml}$ of Con A. The right panel of this figure shows the dose–response relationship for the proliferation of mouse splenic T cells in the range 2–30 $\mu\text{g}/\text{ml}$ of Con A, with the peak stimulation occurring at a concentration of 10 $\mu\text{g}/\text{ml}$ of Con A. It should be noted that the amplitude of the maximum stimulation in the case of splenic T lymphocytes is much higher, doubling that of the response of thymocytes. It is interesting to note that by addition of 5×10^{-12} M of corticosterone (CS), a subphysiological concentration of the hormone (the physiological range being $10\text{--}30 \times 10^{-8}$ M), the stimulating effect of different concentrations

FIGURE 1 Dose-response curves of Con A-induced proliferation of thymic (left panel) and splenic (right panel) lymphocytes. $M \pm \text{SE}$, $n = 3$ for each dose point.

of Con A on the proliferation of both the thymocytes and splenic T lymphocytes is further potentiated, with the most significant augmentation of proliferation occurring at a concentration of $5 \mu\text{g/ml}$ of Con A in both cases (Figure 2, unpublished data). This concentration of Con A ($5 \mu\text{g/ml}$) is sub-optimal since the maximal stimulation of thymic and splenic lymphocytes occurred at the concentrations of 10 and $15 \mu\text{g/ml}$, respectively, as shown in Figure 1.

FIGURE 2 Dose-response curves of Con A-induced proliferation of thymic (left panel) and splenic (right panel) lymphocytes in the presence of low-level CS. $M \pm \text{SE}$, $n = 3$ for each dose point; in both cases the presence of CS 5×10^{-12} M in the culture further increased the lymphocyte proliferation induced by $5 \mu\text{g/ml}$ of Con A ($p < 0.01$).

EFFECT OF RADIATION ON HYPOTHALAMIC–PITUITARY–ADRENOCORTICAL AXIS

It has long been recognized that the hypothalamic–pituitary–adrenocortical (HPA) axis has a profound influence on the regulation of the immune system (Besedovsky and Sorkin, 1979; Besedovsky and Rey, 1996; Haddad *et al.*, 2002). Under physiological conditions, the HPA axis exerts a (tonic) repression on the immune system, preventing its overaction, especially on the growth and function of the thymus (Hadden, 1998; Sheridan *et al.*, 1998). Hyperfunction of the HPA axis would lead to atrophy of the thymus and correspondingly suppression of immune functions. On the other hand, a lowering of the hormonal level of the end organ of this axis (i.e., use of serum corticosteroids) would have an opposite effect. We tested this by adding different concentration of CS to cultured splenic and thymic lymphocytes in a medium containing 5 $\mu\text{g}/\text{ml}$ Con A. It was found that a concentration of corticosterone at 10^{-11} M would stimulate lymphocyte proliferation and a concentration of corticosterone at 10^{-7} M would lead to its suppression (Figure 3) (Liu *et al.*, 1993, 1994a). There is lowering of the activities of the HPA axis after whole-body X-irradiation of mice with 0.075 Gy, expressed as lowered serum concentrations of both ACTH and CS (Figure 4) (Liu *et al.*, 1994b). It can be seen in this figure that the ACTH and CS levels decreased by more than 30 and 70%, respectively, 1 day after irradiation. The mRNA level of the proopiomelanocortin (*POMC*) gene in the hypothalamus decreased early in the first few hours after low-dose radiation (inset at the upper right corner of Figure 4) (Wan and Liu, 1998). This change implicates a down-regulation of the hypothalamic control of the hypophyseal

FIGURE 3 Dose–response curve of mouse thymic and splenic T lymphocyte proliferation after exposure to CS. Thymic and splenic lymphocytes were incubated in the presence of 5 $\mu\text{g}/\text{ml}$ of Con A and different concentrations of corticosterone were added to act for 24 h. Corticosterone caused significant stimulation of proliferation at 10^{-11} M and suppression at 10^{-7} M.

FIGURE 4 Mouse serum ACTH and CS levels after whole-body X-irradiation with 0.075 Gy. $n = 4-6$ for each dose point; control values of mouse serum ACTH and CS were 655.6 ± 50.2 ng/L and 47.1 ± 7.6 μ g/L, respectively. Inset at upper right corner: mRNA level of *POMC* gene in mouse hypothalamus measured with *in situ* hybridization after whole-body X-irradiation with 0.075 Gy (representative data from two parallel experiments).

function, since the *POMC* gene in the hypothalamus regulates ACTH and corticosteroid secretion (Raffin-Sanson *et al.*, 2003; Krude *et al.*, 2003).

GLUCOCORTICOID RECEPTORS AS SIGNAL MOLECULES

An important aspect of the hormonal control of immune functions is the regulation of signal transduction in the immune cells. The action of corticosteroids is realized via the cytoplasmic glucocorticoid receptor (GCR), which binds the hormone to form a complex that is transported to the nucleus, where it activates gene induction after binding of this complex with the glucocorticoid response element in the DNA molecule. Normally GCR exists in the cytoplasm as a complex with heat shock protein (HSP), and binding of glucocorticoid with GCR releases the latter from HSP (Gehring, 1993; Hutchinson *et al.*, 1993). It was reported that low-dose radiation increased the level of HSP in mouse lymphocytes (Nogami *et al.*, 1993). Consequently, there exists the possibility of increased binding of HSP with GCR after low-dose radiation, which would result in its decreased entry into the nucleus. At the same time, low-dose radiation decreases the number of GCRs in splenic T cells whereas high-dose radiation increases it (Figure 5) (Duan *et al.*, 1992). This would be another factor that would further decrease the entry of GCR into the nucleus to activate transcription.

FIGURE 5 Dose–response curve of GCR in splenocytes 24 h after whole-body X-irradiation. $M \pm SE$, $n = 6$ for each dose point; ^3H -dexamethasone was used in measuring splenocyte GCR.

CALCIUM SIGNALING

Intracellular calcium mobilization occupies an important position in the signal pathways in Con A–induced proliferation, both under normal conditions (Isakov and Altman, 1985; Sei and Arora, 1991) and after low-dose radiation (Liu *et al.*, 1994c; Liu, 2003). Whole-body X-irradiation with 0.075 Gy further accentuates this Con A–induced calcium mobilization (Figure 6) (Liu, 1998, 2003), which is accompanied by increased expression of its downstream molecule calcineurin (Figure 6).

The further augmentation by low-dose radiation of both the Con A–induced increase in calcium mobilization and up-regulated expression of

FIGURE 6 Time course of molecular changes related to mouse thymocyte proliferation after whole-body exposure to 0.075 Gy X-rays. Error bars omitted for clarity; $n = 4\text{--}6$ for each dose point.

calcineurin reaches a peak 24 h after low-dose radiation. This coincides with the up-regulation in CD3 expression and the rise of cGMP content in the thymocytes (Figure 6), and also with the decrease in serum CS level and cytoplasmic GCR number (Figures 4 and 5, respectively) (Liu, 1998, 2003). These changes imply that intracellular calcium mobilization may act in concert with other intracellular signal molecules and neurohormonal changes induced by low-dose radiation.

THE PINEAL GLAND AND MELATONIN

Besides the immunoregulatory effect of the HPA axis, the pineal gland was found to be an organ exerting profound influence on immunity (Pierpaoli, 1998; Raffin-Sanson *et al.*, 2003). Studies on the influence of melatonin on low-dose radiation effects have demonstrated that the pineal gland might be an important regulator and melatonin an important signal molecule in the mechanism of low-dose radiation-induced hormesis (Liang *et al.*, 1999). It was found that whole-body X-irradiation with 0.075 Gy caused changes in the pineal gland, including increases in DNA synthesis, decreases in apoptosis as well as a rise of melatonin and cGMP content in the gland (Li *et al.*, 2001, 2002). In pinealectomized (with a laser beam) mice exposed to low-dose radiation there was an elevation of serum CS (Figure 7) instead of the decrease observed in intact mice exposed to low-dose radiation as shown in Figure 4. At the same time, the proliferative capability of the thymocytes and splenic

FIGURE 7 Effect of whole-body X-irradiation with 0.075 Gy on serum CS level in pinealectomized mice. Mice were pinealectomized with a laser beam and whole-body X-irradiation was given 3 days after pinealectomy. Groups of irradiated mice ($n = 6$ for each group) were decapitated at 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 72 h after irradiation. Serum samples (including those from sham-irradiated intact and pinealectomized controls) were kept at -70°C before determination of CS content using competitive protein binding assay.

T cells was markedly depressed after exposure of pinealectomized mice to low-dose radiation. The replacement of melatonin *in vivo* (injection of melatonin to pinealectomized mice) or *in vitro* (addition of melatonin to cultured splenic lymphocytes obtained from pinealectomized mice) could restore the increase in proliferative response induced by low-dose radiation (Liang *et al.*, 1999).

NEUROTRANSMITTERS

The influence of neurotransmitters studied includes the effect of acetylcholine and catecholamines. It is generally believed that epinephrine (E) and norepinephrine (NE) augment the activity of granulocytes while acetylcholine enhances the activity of T lymphocytes, since granulocytes bear adrenergic receptors whereas T cells bear cholinergic receptors (Abo and Kawamura, 2002). Our experiments showed that acetylcholine increased the proliferation of normal T lymphocytes in a wide range of concentrations ($1\text{--}533 \times 10^{-9}$ M) and could further increase the already up-regulated T-cell proliferation induced by low-dose radiation in concentrations above 22×10^{-9} M (Zhao *et al.*, 1996). However, after *in vitro* low-dose radiation the T-cell proliferation was further up-regulated only by the highest concentration of acetylcholine (533×10^{-9} M).

It was also found that E and NE in concentrations of $10^{-9}\text{--}10^{-7}$ M could significantly increase the proliferative reaction of splenic lymphocytes to Con A, and E and NE at 10^{-7} M further augmented the up-regulated proliferative activity induced by low-dose radiation (Figure 8) (Liu *et al.*, 1993, 1994a). It was observed that E, NE, and CS, each at a concentration of 10^{-10} M, showed no effect on proliferation of thymocytes and splenic T cells.

FIGURE 8 Effect of NE on proliferation of thymic (left panel) and splenic (right panel) lymphocytes induced by Con A. $M \pm SE$, $n = 3$ for each dose point; in both cases the presence of NE 10^{-7} M in the culture further increased the lymphocyte proliferation induced by 5 and 10 $\mu\text{g/ml}$ of Con A ($p < 0.01$).

It is interesting to note that E+CS or NE+CS each in a concentration of 10^{-10} M could up-regulate the proliferative activity of the thymocytes and splenic T cells from normal as well as 0.075-Gy-irradiated mice. If E+NE+CS each in a concentration of 10^{-10} M were added to thymocytes and splenic T cells from either normal or low-dose irradiated mice, the proliferative activity was stimulated to an even higher degree. The magnitude of augmentation of cell proliferation was higher than that induced by addition of the calcium ionophore A23187 ($1 \mu\text{g/ml}$) or the PKC agonist PMA (5 ng/ml) to cell cultures from normal as well as low-dose irradiated mice, as illustrated in Figure 9 (Liu *et al.*, 1994a). Whole-body X-irradiation of mice with

FIGURE 9 Effect of E, NE plus CS as well as A23187 and phorbol myristate acetate (PMA) on Con A-induced proliferation of thymocytes and splenic T cells from normal and 0.075 Gy-irradiated mice. (A) splenic T cells; (B) thymocytes; $M \pm SD$, $n = 3$ for each time point. Epinephrine (E), norepinephrine (NE), and corticosterone (CS) each at a concentration of 10^{-10} M acting together, as well as A23187 at $1 \mu\text{g/ml}$ and phorbol myristate acetate (PMA) at 5 ng/ml increased the proliferation of lymphocytes induced by $5 \mu\text{g/ml}$ of Con A in normal (cells harvested from sham-irradiated mice, "zero time") and irradiated mice (cells harvested 1, 4, and 7 days after exposure).

0.075 Gy only slightly increased splenic NE (unpublished data). These observations might imply that, under a subphysiological concentration of CS in the external environment of thymocytes and splenic T cells, slight changes in E and NE concentrations could significantly affect the Ca^{2+} signaling and PKC pathways in the cells. Such experimental data suggest that systemic regulation may exert a profound influence on immune activation after low-dose radiation.

FURTHER WORK NEEDED

The neuroendocrine regulation of immunity is a very complex process. The concept of a neuro–endocrine–immune axis denotes that each constituent part of the axis may influence the function of the other via neurotransmitters, hormones, cytokines, and chemokines, and that neuroendocrine cells may produce cytokines and chemokines, and immune cells may produce neurotransmitters and hormones under specific conditions (Grimm *et al.*, 1998; Haddad *et al.*, 2002). Therefore, the whole picture of neurohormonal regulation in radiation-induced immune changes may be much more complicated than currently believed. More work on the dose–response relationship of the effect of different mediators of the neuroendocrine system on immunity, as well as changes in their tissue content, after exposure may improve our understanding of the nature of low-dose radiation-induced hormesis in the intact organism.

REFERENCES

- Abo T, Kawamura T. Immunomodulation by the autonomic nervous system: Therapeutic approach for cancer, collagen diseases, and inflammatory bowel diseases. *Therap Apher*. 2002;6:348–357.
- Alimkhodzhaeva PR, Usmanova AS, Gil'dieva BS. Effect of hydrocortisone on spontaneous and mitogen-dependent activity of peripheral blood lymphocytes in some vertebrates. *Bull Exp Biol Med*. 2002;133:471–474.
- Besedovsky HO, Rey A. Immune-neuro-endocrine interactions: Facts and hypotheses. *Endocr Rev*. 1996;17:64–102.
- Besedovsky HO, Sorkin E. Net work of immune-neuro-endocrine interactions. *Clin Exp Immunol*. 1979;27:1–12.
- Duan X, Zhang M, Liu SZ. Studies on the interrelationship of serum corticosterone content and glucocorticoid receptor changes in spleic cells after X-irradiation of rats. *J N Bethune Univ Med Sci*. 1992;18:5–8.
- Gehring U. The structure of glucocorticoid receptors. *J Steroid Biochem Mol Biol*. 1993;45:183–190.
- Goytia-Acevedo RC, Cebrian ME, Calderon-Aranda ES. Differential effects of arsenic on intracellular free calcium levels and the proliferative response of murine mitogen-stimulated lymphocytes. *Toxicology*. 2003;189:235–244.
- Grimm MC, Ben-Baruch A, Taub DD, Howard OMZ, Wang JM, Oppenheim JJ. Opiate inhibition of chemokine-induced chemotaxis. *Ann NY Acad Sci*. 1998;840:9–20.
- Haddad JJ, Saade NE, Safieh-Garabedian BJ. Cytokines and neuro-immune-endocrine interactions: A role for the hypothalamic-pituitary-adrenal revolving axis. *J Neuroimmunol*. 2002;133:1–19.
- Hadden JW. Thymic endocrinology. *Ann NY Acad Sci*. 1998;840:352–358.
- Hutchinson KA, Sherrer IA, Stancota LF. Regulation of glucocorticoid receptor function through assembly a receptor-heat shock protein complex. *Ann NY Acad Sci*. 1998;684:35–48.

- Isakov N, Altman A. Tumor promoters in conjunction with calcium ionophores mimic antigenic stimulation by reactivation of alloantigen-primed murine T lymphocytes. *J Immunol.* 1985;135:3674–3680.
- James SJ, Makinodan T. T cell proliferation in normal and autoimmune-prone mice after extended exposure to low doses of ionizing radiation and/or caloric restriction. *Int J Radiat Biol.* 1988;53:137–152.
- Krude H, Biebermann H, Gruters A. Mutations in the human proopiomelanocortin gene. *Ann NY Acad Sci.* 2003;994:233–239.
- Leon MA. Application of homogeneous macromolecules with defined specificities in studies of cell-dependent biological systems. In: Cohen S, Cudkowicz G, McCluskey RT. *Cellular Interactions in the Immune System.* S Karger; 1971:231–234, Basel.
- Li XF, Chi BR, Ma L, Zhang M. Effect of low dose radiation of cell cycle in pineal gland. *J N Bethune Univ Med Sci.* 2001;27:502–503.
- Li XF, Ma L, Zhang M, Chi BR. Effect of low dose radiation on cGMP level of pineal gland in mice. *J Radiat Res Radiat Proc.* 2002;20:158–160.
- Liang Y, Zhang M, Liu SZ. Studies on the regulatory role of pineal gland in radiation hormesis of the immune system. *Chin J Pathophysiol.* 1999;15:938–940.
- Liu SZ. Biological defense and adaptation induced by low dose radiation. *Hum Ecol Risk Assess.* 1998;4:1217–1254.
- Liu SZ. Non-linear dose-response relationship in the immune system following exposure to ionizing radiation: Mechanisms and implications. *Nonlin Biol Toxicol Med.* 2003;1:71–92.
- Liu SZ, Han ZB, Liu WH, Ju GZ, Qi J. Modulatory effect of catecholamines and corticosterone on proliferative activity of thymic and splenic lymphocytes. *J N Bethune Univ Med Sci.* 1993;19:219–223.
- Liu SZ, Han ZB, Liu WH. Changes in lymphocyte reactivity to modulatory factors following low dose ionizing radiation. *Biomed Environ Sci.* 1994a;7:130–135.
- Liu SZ, Zhao Y, Han ZB, Gong SL, Zhang M, Liu WH. Role of changes in hypothalamic-pituitary-adrenocortical axis in the mechanism of immunoenhancement induced by low dose radiation. *Chin J Radiol Med Prot.* 1994b;14:11–14.
- Liu SZ, Su X, Zhang YC, Zhao Y. Signal transduction in lymphocytes after low dose radiation. *Int J Occup Med Toxicol.* 1994c;3:107–117.
- Makinodan T, James SJ. T cell potentiation by low dose ionizing radiation: Possible mechanisms. *Health Phys.* 1990;59:29–34.
- Nogami M, Huang JT, James SJ. Mice chronically exposed to low dose ionizing radiation possess splenocytes with increased levels of HSP70 mRNA, HSC72 and HSP70 and with an increased capacity to proliferate. *Int J Radiat Biol* 1993;63:775–783.
- Pierpaoli W. Neuroimmunomodulation of aging. A program in the pineal gland. *Ann NY Acad Sci.* 1998;840:491–497.
- Raffin-Sanson ML, de Keyser Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: From physiology to pathological conditions. *Eur J Endocrinol.* 2003;149:79–90.
- Sei Y, Arora PK. Quantitative analysis of calcium (Ca^{2+}) mobilization after stimulation with mitogens or anti-CD3 antibodies. Simultaneous fluo-3 and immunofluorescence flow cytometry. *J Immunol Method.* 1991;137:237–244.
- Sheridan JF, Dobbs C, Jung J, Chu X, Konstantinos A, Padgett D, Glaser R. Stress-induced neuroendocrine modulation of viral pathogenesis and immunity. *Ann NY Acad Sci.* 1998;840:803–808.
- Troup GM, Anderson RE. Radiation-induced augmentation of mitogenic responsiveness in murine splenic cells. *Fed Proc.* 1982;4:335–338.
- Wan H, Liu SZ. Effect of low dose radiation on the transcription level of POMC in the hypothalamus and immune organs. *Chin J Radiol Med Prot.* 1998;18:145–147.
- Zhao Y, Gong SL, Liu SZ. Effect of acetylcholine on proliferative reaction of murine splenocytes exposed to low dose radiation. *J Radiat Res Radiat Proc.* 1996;231–234.