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Growth hormone protects human lymphocytes from irradiation-induced cell death

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1 Undesired effects of cancer radiotherapy mainly affect the hematopoietic system. Growth hormone (GH) participates in both hematopoiesis and modulation of the immune response. We report both r-hGH cell death prevention and restoration of secretory capacities of irradiated human peripheral blood lymphocytes (PBL) *in vitro*.

2 r-hGH induced cell survival and increased proliferation of irradiated cells. Western blot analysis indicated that these effects of GH were paralleled by increased expression of the antiapoptotic protein Bcl-2.

3 r-hGH restored mitogen-stimulated release of IL-2 by PBL. Preincubation of irradiated lymphocytes with the growth hormone receptor (GHR) antagonists B2036 and G120 K abrogated r-hGH-dependent IL-2 release.

4 These results demonstrate that r-hGH protects irradiated PBL from death in a specific, receptor-mediated manner. Such effect of r-hGH on PBL involves activation of the antiapoptotic gene *bcl-2* and prevention of cell death, associated with preserved functional cell capacity. Finally, potential use of GH as an immunopotentiating agent could be envisioned during radiation therapy of cancer.
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Abbreviations: GHR, growth hormone receptor; IL-2, interleukin-2; PBL, peripheral blood lymphocytes; PRL, prolactin; r-hGH, recombinant human growth hormone

Introduction

Biomorphologic changes occurring during acute radiation syndrome may involve the lymphohemopoietic system. For example, fatal septicemia is an untoward effect related to immunosuppression caused by therapeutic or environmental exposure to radiations (Love *et al*., 1998; Hart *et al*., 2001). In fact, progression of radiation-induced atrophy of lymph nodes, spleen and bone marrow enhances susceptibility to infections by opportunistic microorganisms (Anderson & Warner, 1976).

Growth hormone (GH), a peptide molecule that controls somatic development (Neta, 1990), also regulates an array of physiological processes, including hematopoiesis (Bengtsson *et al*., 1990) and the immune response (Miale *et al*., 1996). Interestingly, GH has been shown to improve impaired immune response in genetically (Murphy *et al*., 1993) and pharmacologically (Franco *et al*., 1990) immune-depressed mice.

GH induces dimerization of a single-pass homodimeric transmembrane receptor (GHR) associated with a tyrosine kinase of the Janus kinase family named JAK2 (Argetsinger *et al*., 1993). GHR belongs to the erythropoietin/interleukin-2 (IL-2)/GH receptor superfamily (Bazan, 1989). Cytokines binding to this receptor superfamily, such as granulocyte – macrophage- and granulocyte-colony-stimulating factor display radioprotective effects (Murphy *et al*., 1992a,b; Tisch *et al*., 1998). Evidence indicates that the latter are exerted via the induction of antiapoptotic genes (Liu *et al*., 1999). In this line, binding of GH to its receptor results, for example, in protection from Fas-mediated apoptosis in monocytes through upregulation of the antiapoptotic protein Bcl-2 (Haeffner *et al*., 1999).

GHR dimerization (Chen *et al*., 1991) is prevented by recently characterized GH analogs, which have been shown to behave as specific GHR antagonists (Chen *et al*., 1994). We have recently demonstrated that the two GHR antagonists B2036 and G120 K (Fuh *et al*., 1992; Chen *et al*., 1994) totally abrogate beneficial effects of recombinant human GH (r-hGH) on postirradiation cell death and compromised hormone secretion in primary cultures of rat anterior pituitary cells (Chiarenza *et al*., 2000).

For these reasons, it appeared of interest to assess possible radioprotective effects of r-hGH on *in vitro* human peripheral blood lymphocytes (PBL) irradiated sublethally, by estimating postirradiation residual cell viability. In addition, to elucidate the nature of GH-dependent protective effects, we also studied the expression of the *bcl-2* oncogene. Moreover, to test residual functional activity of irradiated PBL, we investigated whether

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treatment with h-rGH could affect lymphocyte responsiveness to mitogens, either by measuring proliferation rate and IL-2 release.

Finally, we also characterized pharmacologically the effects of GH, using the two potent GHR antagonists B2036 and G120 K (Chen *et al*., 1994).

Methods

Chemicals

All chemicals were purchased from Sigma Chemicals Italia (Milan, Italy), unless otherwise specified. Plasticware was from NUNC (Roskilde, DK). r-hGH was a gift from Serono Pharma S.p.A (Rome, Italy).

The GH antagonists B2036 and hGH G120 K (referred to as G120 K) were kindly provided by Sensus Drug Development Corporation (Austin, TX, USA). These compounds contain various mutations as described earlier (Fuh *et al*., 1992).

Primary cultures of human lymphocytes

A volume of 20 ml of peripheral blood from healthy volunteer donors were diluted 1 : 1 with Ca^{2+}-Mg^{2+}-free phosphate-buffered saline (PBS, pH 7.4; Gibco, Life Technologies Italia, Milan, Italy) and stratified onto a Ficoll – Hypaque gradient (Pharmacia, Piscataway, NJ, USA). Samples were then spun at 3000 rpm for 15 min at room temperature. Resulting buffy coats were separated and washed twice in PBS. The pellets were resuspended in RPMI 1640 medium (Gibco, Life Technologies Italia, Milan, Italy) supplemented with 10% fetal calf serum (FCS, Gibco, Life Technologies Italia, Milan, Italy), 50 μg/ml gentamicin (Gibco, Life Technologies Italia, Milan, Italy), 2 g $NaHCO_3$ (Gibco, Life Technologies Italia, Milan, Italy) and 1 ml/l L-glutamine (Gibco, Life Technologies Italia, Milan, Italy). The volume of cell suspension was then properly adjusted, and cells were plated in a 96-well plate at a density of 2×10^5/well.

Pharmacological treatment of cultures and irradiation procedures

All cells were treated for periods of 12 h with graded concentrations (from 0.1 to 50 nM) of r-hGH either before, after, or before and after irradiation. Cells were irradiated with a single 8 Gy dose produced by a ^{60}Co source. After irradiation, remaining cells were treated for 24 h as follows: postirradiation treatment with GH for 12 h plus 12 h incubation with plain medium; or 24 h in plain medium for cultures receiving only preirradiation treatment with GH.

Viability was then evaluated by the trypan blue method and the media frozen at -80°C until processed for IL-2 ELISA.

Apoptosis and proliferation assays

For quantitation of apoptosis, cells were stained with Hoechst 33258 (Darzynkiewicz *et al*., 1994) and nuclear morphological changes, such as chromatin condensation and fragmentation, were examined under a fluorescent microscope (Leica). The number of apoptotic cells was counted over a total cell number $>10^3$ and expressed as percent.

Cell proliferation was evaluated by means of the routine tritiated thymidine method (^{3}H-Thy, specific activity: 25 Ci mmol^{-1}; Amersham Pharmacia Biotech Italia, Cologno Monzese, Italy).

Western blot analysis

After separation and collection of PBL from peripheral blood, cells were cultured in 60-mm plastic Petri dishes and treated as follows: r-hGH, r-hGH plus GHR antagonist B2036 or antagonist B2036 alone 12 h before irradiation. At 12 h following treatment, cells were solubilized in Laemmli buffer, and 80 μg of proteins separated by SDS – PAGE (12%), transferred on to a nitrocellulose sheet, probed with an anti-rabbit/human Bcl-2 Ab (1 : 1000; Alexis Biochemicals, San Diego, USA) and with the secondary peroxidase-conjugated anti-rabbit Ab (1 : 1000; Amersham Italia S.r.l., Milan, Italy), which was then detected by enhanced chemiluminescence (ECL; Amersham Italia S.r.l., Milan, Italy).

Phytohemagglutinin proliferation test

Stimulation test with phytohemagglutinin (PHA) was performed in irradiated human PBL cultures treated with 5 nM r-hGH before and after irradiation according to the above-described protocol. The media were aspirated 24 h after irradiation, and cells incubated for an additional 24 h with fresh complete medium containing 5 μg/ml of PHA. In other experiments, B2036 or G120 K (50 nM, a concentration in 10-fold excess to ensure efficient competition with GH) (Fuh *et al*., 1992) was added 15 min before treatment with r-hGH.

After 24 h, media were separated by centrifugation (1500 rpm) and frozen at -80°C until assayed for IL-2.

Media IL-2 assay

IL-2 was measured in tissue culture media by means of the commercial Quantikine ELISA kit according to the manufacturer's instructions (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany).

IL-2 secretion in unstimulated, untreated, and nonirradiated cells was taken as basal secretion value. To normalize results, IL-2 secretion was calculated in picograms per milligram (pg/mg) of cell proteins. Protein concentration was determined by the Bradford dye-binding procedure (Bradford, 1976).

Statistical analysis of results

All experiments were run in triplicate and repeated at least twice on different days. Statistical analysis of results was performed using the one- or two-way analysis of variance (one-way ANOVA test), followed by Duncan's multiple range test. Significance was set for $P<0.05$.

Results

Effect of r-hGH on viability of irradiated human PBL

We assessed the effects of treatment with r-hGH on lethality resulting from irradiation of cultured human PBL. Incubation of irradiated cells with h-rGH resulted in partial prevention of cell death.

Percent of cell survival was significantly higher in cultures treated with r-hGH (concentration range: 0.1–50 nM) 12 h before irradiation. The effect of r-hGH was concentration-dependent. Maximal effect occurred at a concentration of 5 nM (EC_{50}: 2.8 nM). Protective effect of GH upon irradiation-induced cell death was not observed in cultures treated with r-hGH 12 h after irradiation.

Treatment of human lymphocytes with r-hGH 12 h before and 12 h after irradiation resulted in significantly increased cell survival. The effect of r-hGH was concentration dependent. Maximal effect of r-hGH was at a concentration of 5 nM (EC_{50}: 1.1 nM) (Figure 1). All untreated cells showed reduced survival.

In order to verify whether the observed survival effect of GH on lymphocytes was rather due to decreased cell death than to increased cell proliferation, we assessed the apoptosis rate of cells by the Hoechst staining method. Treatment with GH (5 nM, 12 h before and after) rescued cells from apoptosis caused by sublethal irradiation (about 70% of cells), as indicated by the significantly decreased number of apoptotic cells (Table 1).

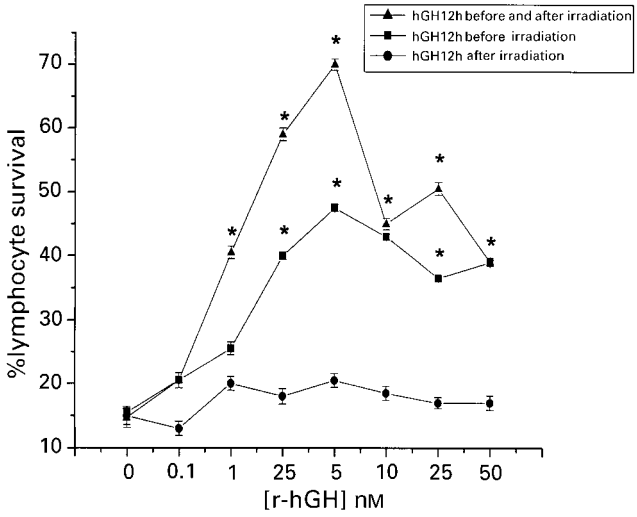

Figure 1 Percent survival in irradiated primary cultures of human PBL treated with r-hGH (concentration range: 0.1 – 50 nM), either 12 h before irradiation (squares), 12 h after irradiation (circles), or 12 h before and after irradiation (triangles). Cells were exposed to 8 Gy radiation, produced by a ^{60}Co source. Vertical bars are the means ± s.e. (*$P<0.05$, ANOVA + Duncan's multiple range test).

Effects of r-hGH on the expression of the antiapoptotic protein Bcl-2 in irradiated human PBL

In order to verify whether the effect of r-hGH could affect apoptosis-related mechanisms, we also studied the expression of the antiapoptotic protein Bcl-2 following 12 h treatment with h-rGH.

Western blot analysis showed a significant increase of Bcl-2 in irradiated PBL treated with r-hGH 12 h prior to irradiation. A similar effect occurred in intact lymphocytes (Figure 2, panel a).

The GHR antagonist B2036 prevented GH-dependent increase in both intact (Figure 2, panel b) and irradiated PBL (Figure 2, panel c).

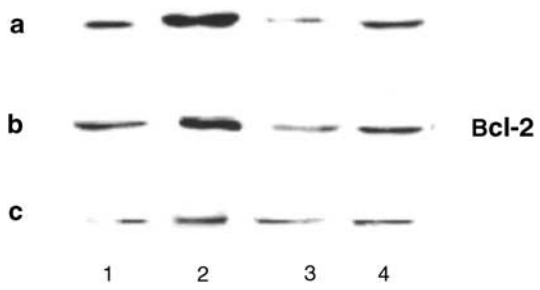

Figure 2 Panel a: Western blot analysis of Bcl-2 protein expression in PBL treated as follows: lane 1: intact untreated cells; lane 2: intact cells treated with r-hGH; lane 3: irradiated untreated cells; lane 4: irradiated cells treated with r-hGH; Panel b: lane 1, intact untreated cells; lane 2, intact cells treated with r-hGH; lane 3, intact cells treated with the GHR antagonist B2036; lane 4, intact cells treated with r-hGH plus the GHR antagonist B2036. Panel c: lane 1, irradiated untreated cells; lane 2, irradiated cells treated with r-hGH; lane 3, irradiated cells treated with the GHR antagonist B2036; lane 4, irradiated cells treated with r-hGH plus the GHR antagonist B2036.

Effects of r-hGH on proliferation of irradiated human PBL

To verify residual proliferating capacity of irradiated PBL, we evaluated the rate of ^{3}H-Thy incorporation following treatment with r-hGH.

r-hGH stimulated proliferation of intact cultured human lymphocytes in a concentration-related manner. Peak of the effect occurred at a concentration of 10 nM.

Table 1 Rescue from irradiation-induced apoptosis in cultured human PBL treated with r-hGH[a]

Percent apoptotic cells						
Intact	*Irradiated*	*GH before*	*GH after*	*GH bef+aft*	*GH+B2036*	*B2036*
0.95 ± 0.1	79.9 ± 4.3	44.1 ± 5.2*	61.3 ± 6.9*	29.7 ± 5.6*	69.9 ± 5.3	72.3 ± 8.1

[a]Nonirradiated or irradiated PBL were treated with 5 nM r-hGH at different times (12 h after or before, or before and after irradiation). Effects of GH were characterized by using the GHR antagonist B2036 (50 nM). Typical apoptotic cells stained with Hoechst 33258 were counted among $>10^3$ cells and percent cell apoptosis was determined. Data are means ± s.e. from three independent experiments, each performed in triplicate. Experiments with B2036 were performed in cultures treated with r-hGH before and after irradiation. *$P<0.05$ vs irradiated (untreated; ANOVA followed by Duncan's test).

Incubation with r-hGH for 12 h prior to irradiation determined a significant increase of proliferation of irradiated PBL. The effect of r-hGH was concentration-dependent. Peak of r-hGH effect occurred at 5 nM (EC_{50}: 3.5 nM).

Treatment of irradiated human PBL with r-hGH for 12 h after irradiation resulted in a significant increase of cell proliferation compared to untreated cells (EC_{50}: 4.2 nM).

Treatment of irradiated human PBL with r-hGH 12 h prior to and after irradiation resulted in an increased cell proliferation compared to either untreated cells and other groups of treatment, as indicated by the relative EC_{50} s (1.3 nM). The effect of r-hGH was concentration dependent. Maximal effect of r-hGH occurred at a concentration of 5 nM (Figure 3, lower panel).

In additional experiments aimed to understand the relevance of such effect of GH, we compared the mitogenic potency of r-hGH (5 nM) *versus* PHA (5 μg ml^{-1}) in intact human PBL. Both substances exerted comparable effects upon PBL proliferation. Incubation with both r-hGH and PHA resulted in an additive stimulatory effect on PBL proliferation (Figure 3, upper panel).

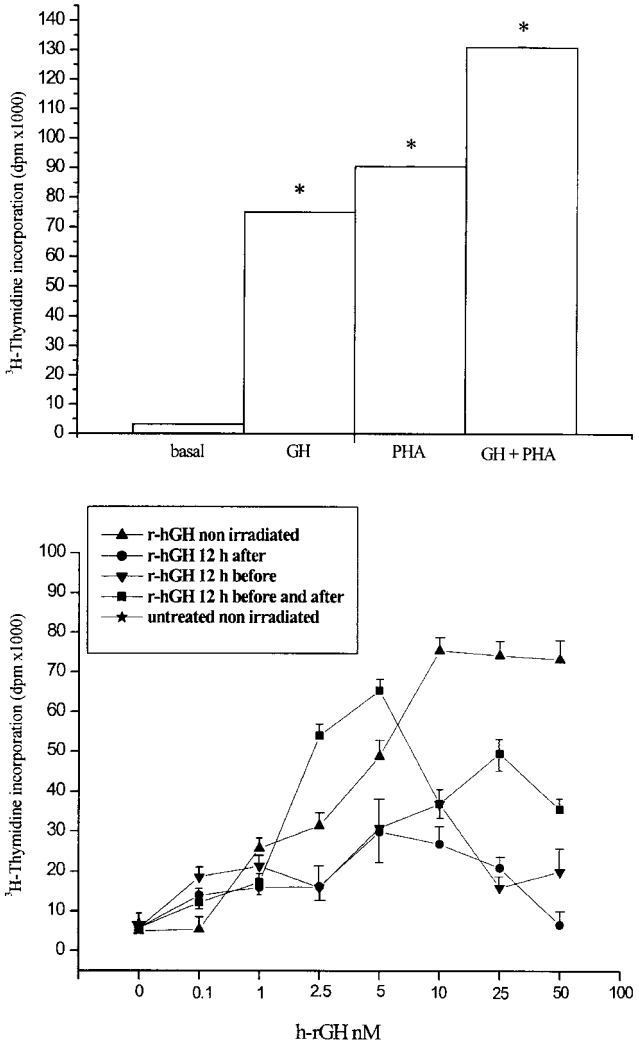

Figure 3 Effects of r-hGH on thymidine incorporation in human PBL *in vitro*. Upper panel: Effects of r-hGH (5 nM), PHA (5 μg/ml) or their combination *vs* basal proliferation in intact primary cultures of human lymphocytes. Vertical bars are the means ± s.e. (*$P<0.05$, ANOVA + Duncan's multiple range test). Lower panel: ^{3}H-Thymidine incorporation into irradiated human lymphocytes treated with r-hGH (concentration range: 0.1 – 50 nM) at different times. Cells were exposed to 8 Gy radiation, produced by a ^{60}Co source. Vertical bars are the means ± s.e. (*$P<0.05$, two-way ANOVA).

Effects of r-hGH and its receptor antagonists on IL-2 released by irradiated human PBL

To assess protective effects of r-hGH upon postirradiation cell function deficit, we measured IL-2 release in the culture media from intact or irradiated human PBL incubated with PHA.

Treatment of irradiated PBL with r-hGH (5 nM) prior to and after irradiation resulted in partially preserved capability of the cells of secreting substantial amounts of IL-2, compared to IL-2 levels detected in the media from untreated, irradiated PBL (Figure 4, upper panel).

The basal value of IL-2 (untreated, nonirradiated PBL) was 27.6 ± 1.4 pg mg^{-1} protein (mean ± s.e.). On the other hand, IL-2 levels were undetectable in irradiated, untreated cells.

To characterize the specificity of the effects of GH, both intact and irradiated lymphocytes were preincubated with each of the two specific GHR antagonists B2036 and G120K. Experiments were conducted in the absence or in the presence of the mitogen PHA.

The GHR antagonist B2036 completely prevented GH-stimulated release of IL-2 in either intact and irradiated lymphocytes in the absence of PHA (Figure 4, upper panel). Similar results were obtained with the GHR antagonist G120K (data not shown). Both GHR antagonists did not show any intrinsic activity.

Irradiated cells preincubated with GHR antagonists and then treated with GH failed to respond to PHA with IL-2 secretion. Both B2036 and G120 K GHR antagonists did not show any intrinsic activity when used alone. In addition, both antagonists failed to inhibit IL-2 release from intact lymphocytes stimulated with mitogenic concentrations of PHA (Figure 4, lower panel).

Discussion

We have shown that treatment with r-hGH significantly reduces postirradiation lethality in human PBL. Such a finding is in accordance with the trophic effects of GH either on B cells (Geffner, 1997), which respond to the hormone with increased proliferation in normal animals (Kimata & Yoshida, 1994), as well as on T cells (Postel-Vinay *et al*., 1997). Interestingly, GH is able to induce proliferation of T cells in genetically immunodeficient DW/J dwarf mice (Murphy *et al*., 1992a,b), a model that resembles the irradiation-dependent immune suppression.

In fact, ionizing radiations induce apoptosis in cells of the immune system (Boreham *et al*., 2000), as shown by increased expression of proapoptotic genes in irradiated human lymphoblastic cells (Yu & Little, 1998).

Here we found that GH rescues cells from apoptosis when added to the cultures prior to and after irradiation. In accordance, we report that treatment of irradiated cells with GH results in increased expression of the antiapoptotic gene *bcl-2*. Increased expression of antiapoptotic genes has been described after treatment of human monocytes with GH (Haeffner *et al*., 1999), reinforcing the concept that GH, at

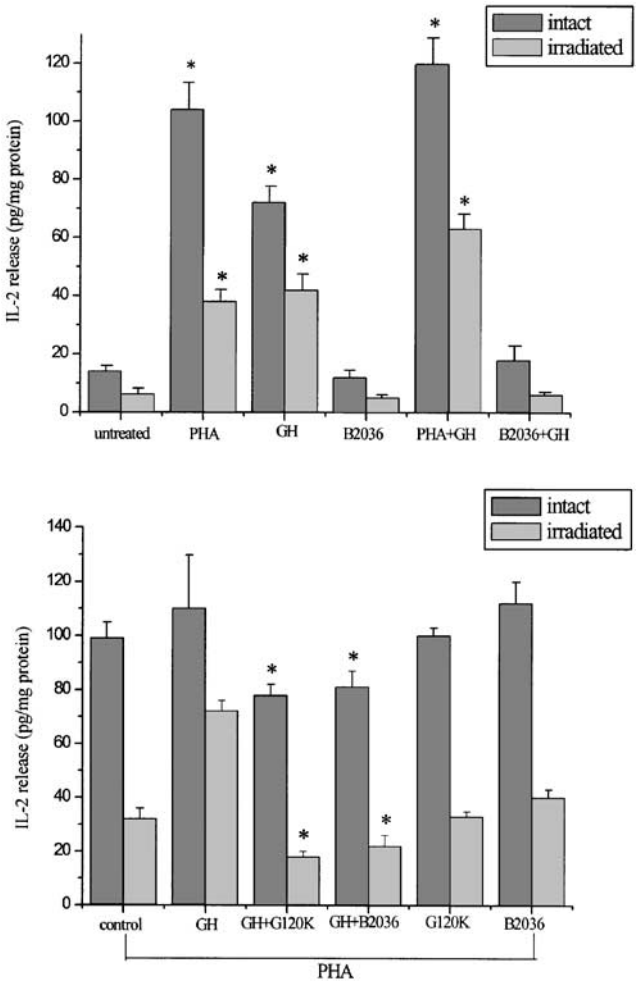

Figure 4 Effects of r-hGH on IL-2 release from irradiated human PBL *in vitro*. Upper panel: Effects of either r-hGH (12 h before and after irradiation), the GHR antagonist B2036, or the mitogen PHA and various combinations, on IL-2 release from either intact or irradiated primary cultures of human PBL. Cells were exposed to 8 Gy radiation produced by a ^{60}Co source. Vertical bars are the means ± s.e. (*$P<0.05$ vs respective groups of untreated cells; ANOVA, followed by Duncan's multiple range test). Lower panel: Effects of the two GHR antagonists G120 K and B2036 (50 nM) on IL-2 release from either intact or irradiated primary cultures of human PBL stimulated with 5 μg/ml PHA and treated with r-hGH (5 nM) 12 h before and after irradiation. Cells were exposed to 8 Gy radiation produced by a ^{60}Co source. Vertical bars are the means ± s.e. (*$P<0.05$ vs respective groups of cells treated with GH; ANOVA, followed by Duncan's multiple range test).

least in part, exerts its protective effects on leukocytes (Jeay *et al*., 2000) via an antiapoptotic mechanism. Interestingly, GH shares its antiapoptotic properties with other peptides binding to the same GH-PRL/CSF/IL-2 receptor superfamily (Bazan, 1989). In addition, GH exerts positive effects upon cell cycle machinery (Yang *et al*., 1999), and, moreover, self-activation of GHR-dependent MAPKK (Vesely, 1981; Liu *et al*., 1999) has also been described in irradiated cancerous cells (Dent *et al*., 1999). Thus, it could be plausible to hypothesize that radioprotective effects of GH are because of multiple mechanisms, all concurring to lymphocyte survival.

IL-2 released by irradiated lymphocytes in response to mitogenic stimuli was significantly decreased. In fact, reduction of functional capacity of surviving lymphocytes has been shown to be another consequence of irradiation (Tisch *et al*., 1998). It is of interest to note that r-hGH was able to restore partially irradiated PBL capacity to release IL-2 in response to mitogenic stimuli, a result corroborated by the additive effect of GH in combination with PHA. Similarly, we have previously reported that treatment with GH substantially restores decreased secretory function of irradiated rat dispersed anterior pituitary cells and stimulated their respective secretagogue factors (Chiarenza *et al*., 2000).

Interestingly, the selective GHR antagonists B2036 and G120 K abolished IL-2 release from irradiated PBL stimulated with PHA and treated with GH.

Human lymphocytes are known to respond to GH by means of specific surface receptors and to respond to their activation with increased proliferation and synthesis (Postel-Vinay *et al*., 1997).

In consideration of the pharmacological activity of r-hGH, usually occurring in the nanomolar range (Vesely, 1981), it is also likely that the beneficial effects of GH on irradiated lymphocytes are mediated by specific GHRs, an hypothesis that is corroborated by inhibition of GH effects in the presence of its receptor antagonists.

Finally, it may be argued that the effects of GH could be mediated by PRL receptors, known to bind GH (Uckun *et al*., 1990) and also expressed by human lymphocytes (Goffin *et al*., 1996). In this line, it has been shown that GHR antagonists used in our study are unable to bind PRL receptors (Goffin *et al*., 1999).

In summary, we have shown that GH partially prevents cell death subsequent to irradiation in human PBL, and restores PBL responsiveness to mitogenic stimuli and secretory capacity via a specific GHR-mediated mechanism.

Our data corroborate the evidence that GH is a trophic factor for the cells of the immune system (Yang *et al*., 1999), and suggest that treatment with GH during irradiation may contribute to maintain efficacy of the immune response (Murphy & Longo, 2000). In the light of our results, GH could represent a valid addition to the current strategies for the prevention of irradiation-related immune system impairment.

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References

ANDERSON, R.E. & WARNER, N.L. (1976). Ionizing radiation and the immune response. *Adv. Immunol.,* **24,** 215 – 335.

ARGETSINGER, L.S., CAMPBELL, G.S. & YANG, X. *et al.* (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell,* **74,** 237 – 244.

BARBERA, N., PALMUCCI, T., CHIARENZA, A., BARTOLONI, G., CORDARO, S., GRECO, S., SCAPAGNINI, U. & BERNARDINI, R. (1993). Radioprotective effects of the association thymopentin-interleukin 1 alpha in the C57BL/6 mouse. *Pharmacol. Toxicol.,* **72,** 256 – 261.

BAZAN, F. (1989). A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors, and p75 IL-2 receptor β chain. *Biochem. Biophys. Res. Commun.,* **164,** 788 – 795.

BENGTSSON, B.A., BRUMMER, R.J. & BOSAEUS, I. (1990). Growth hormone and body composition. *Horm. Res.,* **33,** 19 – 24.

BOREHAM, D.R., DOLLING, J.A., MAVES, S.R., SIWARUNGSUN, N. & MITCHEL, R.E. (2000). Dose-rate effects for apoptosis and micronucleus formation in gamma-irradiated human lymphocytes. *Radiat. Res.,* **153,** 579 – 586.

BRADFORD, MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72,** 248 – 254.

CHEN, W.Y., CHEN, N.Y., YUN, J., WAGNER, T.E. & KOPCHICK, J.J. (1994). *In vitro* and *in vivo* studies of antagonistic effects of human growth hormone analogs. *J. Biol. Chem.,* **269,** 15892 – 15897.

CHEN, W.Y., WIGHT, D.C., WAGNER, T.E. & KOPCHICK, J.J. (1991). Functional antagonism between endogenous mouse growth hormone (GH) and a GH analog results in dwarf mice. *Endocrinology,* **129,** 1402 – 1408.

CHIARENZA, A., LEMPEREUR, L., PALMUCCI, T., CANTARELLA, G., AMICO-ROXAS, M., GOFFIN, V., MURABITO, P., MAGRO, G. & BERNARDINI, R. (2000). Responsiveness of irradiated rat anterior pituitary cells to hypothalamic releasing hormones is restored by treatment with growth hormone. *Neuroendocrinology,* **72,** 392 – 399.

COMBY, E., ANDRE, I., TROUSSARD, X., LEBRUN, E., SOLA, B., BALLET, J.J. & LEPORRIER, M. (1999). *In vitro* evaluation of B-CLL cells apoptotic responses to irradiation. *Leuk. Lymphoma,* **34,** 159 – 166.

DARDENNE, M., MELLO-COELHO, V., GAGNERAULT, M.C. & POSTEL-VINAY, M.C. (1998). Growth hormone receptors and immunocompetent cells. *Ann. N.Y. Acad. Sci.,* **840,** 510 – 517.

DARZYNKIEWICZ, Z., LI, X. & GONG, J. (1994). Assay of cell viability: discrimination of cell dying by apoptosis. *Methods Cell Biol.,* **41,** 15 – 38.

DENT, P., REARDON, D.B., PARK, J.S., BOWERS, G., LOGSDON, C., VALERIE, K. & SCHMIDT-ULLRICH, R. (1999). Radiation-induced release of transforming growth factor α activates the epidermal growth factor receptor and mitogen-activated protein kinase pathway in carcinoma cells, leading to increased proliferation and protection from radiation-induced cell death. *Mol. Biol. Cell.,* **10,** 2493 – 2506.

FERRAG, F., LEBRUN, J.J., TOURAINE, P., NAGANO, M., DARDENNE, M. & KELLY, P.A. (1994). Prolactin and the immune system. *Immunomethods,* **5,** 21 – 30.

FRANCO, P., MARELLI, O. & LATTUADA, D. (1990). Influence of growth hormone on the immunosuppressive effect of prednisolone in mice. *Acta Endocrinol.* (*Copenh*)., **123**, 339 – 344.

FUH, G., CUNNINGHAM, B.C., FUKUNAGA, R., NAGATA, S., GOEDDEL, D.V. & WELLS, J.A. (1992). Rational design of potent antagonists to human growth hormone receptor. *Science,* **256,** 1677 – 1680.

GEFFNER, M. (1997). Effects of growth hormone and insulin-like growth factor I on T- and B-lymphocytes. *Acta. Paediatr. Suppl.,* **423,** 76 – 79.

GOFFIN, V., BERNICHTEIN, S., CARRIÈRE, O., BENNETT, W.F., KOPCHICK, J.J. & KELLY, P.A. (1999). The human growth hormone antagonist B2036 does not interact with the prolactin receptor. *Endocrinology,* **140,** 3853 – 3856.

GOFFIN, V., SHIVERICK, K.T., KELLY, P.A. & MARTIAL, J.A. (1996). Sequence function relationships within the expanding family of prolactin, growth hormone, placental lactogen and related proteins in mammals. *Endocr. Rev.,* **17,** 385 – 410.

HAEFFNER, A., DEAS, O., MOLLEREAU, B., ESTAQUIER, J., MIGNON, A., HAEFFNER-CAVAILLON, N., CHARPENTIER, B., SENIK, A. & HIRSCH, F. (1999). Growth hormone prevents human monocytic cells from Fas-mediated apoptosis by up-regulating Bcl-2 expression. *Eur. J. Immunol.,* **29,** 334 – 344.

HART, P.H., GRIMBALDESTON, M.A. & FINLAY-JONES, J.J. (2001). Sunlight, immunosuppression and skin cancer: role of histamine and mast cells. *Clin. Exp. Pharmacol. Physiol.,* **28,** 1 – 8.

JEAY, S., SONENSHEIN, G.E., POSTEL-VINAY, M.C. & BAIXERAS, E. (2000). Growth hormone prevents apoptosis through activation of nuclear factor-kappa B in interleukin-3-dependent Ba/F3 cell line. *Mol. Endocrinol.,* **14,** 650 – 661.

KIESS, W. & GALLAHER, B. (1998). Hormonal control of programmed cell death/apoptosis. *Eur. J. Endocrinol.,* **138,** 482 – 491.

KIMATA, H. & YOSHIDA, A. (1994). Effect of growth hormone and insulin-like growth factor-I on immunoglobulin production by and growth of human B cells. *J. Clin. Endocrinol. Metab.,* **78,** 635 – 641.

LIU, R., ITOH, T., ARAI, K.I. & WATANABE, S. (1999). Two distinct signaling pathway downstream of Janus Kinase 2 play redundant role for antiapoptotic activity of granulocyte – macrophage lony-stimulating factor. *Mol. Biol. Cell,* **10,** 3959 – 3970.

LOVE, D.W., WHATMORE, A.J., CLAYTON, P.E. & SILVA C.M. (1998). Growth hormone stimulation of the mitogen-activated protein kinase pathway is cell type specific. *Endocrinology,* **139,** 1965 – 1971.

MIALE, T.D., SIRITHORN, S. & AHMED, S. (1996). Efficacy and toxicity of radiation in preparative regimens for pediatric stem cell transplantation. *Med. Oncol.,* **13,** 43 – 61.

MURPHY, W.J., DURUM, S.K. & LONGO, D.L. (1992a). Human growth hormone promotes engraftment of murine or human T cells in severe combined immunodeficient mice. *Proc. Natl. Acad. Sci. U.S.A.,* **89,** 4481 – 4485.

MURPHY, W.J., DURUM, S.K. & LONGO, D.L. (1992b). Role of neuroendocrine hormones in murine T cell development. Growth hormone exerts thymopoietic effects *in vivo*. *J. Immunol.,* **149,** 3851 – 3857.

MURPHY, W.J., DURUM, S.K. & LONGO, D.L. (1993). Differential effects of growth hormone and prolactin on murine T cell development and function. *J. Exp. Med.,* **178,** 231 – 236.

MURPHY, W.J. & LONGO, D.L. (2000). Growth hormone as an immunomodulating therapeutic agent. *Immunol. Today* **21,** 211 – 213.

NETA, R. (1990). Radioprotection and therapy of radiation injury with cytokines. *Prog. Clin. Biol. Res.,* **352,** 471 – 478.

NILSSON, A., OHLSSON, C. ISAKSSON, O.G. LINDAHL, A. & ISGAARD, J. (1994). Hormonal regulation of longitudinal bone growth. *Eur. J. Clin. Nutr.,* **48,** S150 – S158.

POSTEL-VINAY, M.C., DE MELLO COELHO, V. & GAGNERAULT, M.C. *et al*. (1997). Growth hormone stimulates the proliferation of activated mouse T lymphocytes. *Endocrinology,* **138,** 1816 – 1820.

TISCH, M., HEIMLICH, F., DANIEL, V., OPELZ, G. & MAIER, H. (1998). Cellular immune defect caused by postsurgical radiation therapy in patients with head and neck cancer. *Otolaryngol. Head Neck. Surg.,* **119,** 412 – 417.

UCKUN, F.M., SOUZA, L., WADDICK, K.G., WICK, M. & SONG, C.W. (1990). *In vivo* radioprotective effects of recombinant human granulocyte colony-stimulating factor in lethally irradiated mice. *Blood,* **75,** 638 – 645.

VESELY, D.L. (1981). Human and rat growth hormones enhance guanylate cyclase activity. *Am. J. Physiol.,* **240,** E79 – E82.

WHEADON, H., ROBERTS, P.J., WATTS, M.J. & LINCH, D.C. (1999). Changes in signal transduction downstream from the granulocyte – macrophage colony-stimulating factor receptor during differentiation of primary hemopoietic cells. *Exp. Hematol.,* **27,** 1077 – 1086.

YAMADA, M., HATO, F., KINOSHITA, Y., TOMINAGA, K. & TSUJI, Y. (1994). The indirect participation of growth hormone in the thymocyte proliferation system. *Cell Mol. Biol.,* **40,** 111 – 121.

YANG, Y., GUO, L., MA, L. & LIU, X. (1999). Expression of growth hormone and insulin-like growth factor in the immune system of children. *Horm. Metab. Res.,* **31,** 380 – 384.

YU, Y. & LITTLE, J.B. (1998). p53 is involved in but not required for ionizing radiation-induced caspase-3 activation and apoptosis in human lymphoblast cell lines. *Cancer Res.,* **58,** 4277 – 4281.

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