

Effects of Treatment with Interleukin-1 Receptor Antagonist on Endogenous Interleukin-1 Levels in Normal and Irradiated Mice

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Summary

The *in vivo* effects of recombinant human interleukin-1 receptor antagonist (rhIL-1Ra) administration on endogenous IL-1 levels in the circulation and conditioned media (CM) from different immunohematopoietic organ/tissues were studied in CBA mice under steady state and postirradiation conditions. In normal mice, constitutive IL-1 levels were demonstrated in the plasma, CM of peritoneal exudate cells and full-thickness skin explants with low or undetectable levels in CM of splenic and bone marrow cell suspensions. In irradiated mice (2 Gy, X rays) on day 3 post exposure a significant increase of IL-1 levels was seen in the circulation and CM of peritoneal exudate cells, with no significantly different levels in postirradiation bone marrow, spleen and skin. After rhIL-1Ra treatment of the animals (2 x 50 µg/mouse, i.p.), significantly elevated IL-1 levels were observed in the skin and CM of peritoneal exudate cells in normal mice, whereas slightly increased levels were detected in CM of splenic cells. The rhIL-1Ra administration in irradiated mice led to decreased IL-1 concentrations in the circulation, and CM of peritoneal exudate cells and skin. The results pointed out the importance of IL-1 secretion and receptor expression in the maintenance of homeostasis in steady state, as well as during recovery after irradiation. Modulatory effects of IL-1Ra on IL-1 production were dependent on basic endogenous IL-1 concentration.

Key words

rhIL-1Ra • IL-1 • In vivo • Steady-state • Irradiation

Introduction

The multifunctional cytokine interleukin-1 (IL-1) exists as two distinct, related isoforms, designated α and β , which recognize the same cell surface receptors and share various activities. IL-1 affects almost every tissue and organ system and is an important mediator of

the host defense response to injury and infection. However, besides the beneficial biological effects, IL-1 can also exert harmful pathophysiological effects and contribute to the pathogenesis of many diseases. Since IL-1 is involved in both protective and proinflammatory processes, the production and activity of IL-1 are closely regulated events. The multiple control pathways include

the naturally occurring inhibitor IL-1 receptor antagonist (IL-1Ra), capable of competing with IL-1 for the occupancy of IL-1 receptors without eliciting detectable response. Acting as a classical antagonist, IL-1Ra modulates proinflammatory effects of IL-1 (Dinarello 1994, 1996a).

Recently published data have indicated, that normal development and healthy survival requires the expression of IL-1Ra (Hirsch *et al.* 1996, Matsukawa *et al.* 1997) and demonstrated that serum levels of IL-1 are related to the endogenous production of IL-1Ra (Hirsch *et al.* 1996). However, the complex regulatory mechanisms involved in the close control over the production and activity which IL-1Ra exerts on IL-1 are still not well understood. The aim of this study was to assess whether exogenous IL-1Ra treatment affects endogenous IL-1 levels under different physiological/pathological conditions of the organism. The *in vivo* effects of recombinant human IL-1Ra (rhIL-1Ra) treatment on endogenous IL-1 (α and β) plasma levels, as well as on the IL-1 (α and β) presence in different organs of the immunohematopoietic system were evaluated in normal and sublethally irradiated CBA mice.

Materials and Methods

Mice and irradiation

The experiments were carried out on inbred male CBA mice (Breeding Facilities of the Institute for Medical Research, Military Medical Academy, Belgrade) weighing 20-22 g. Whole-body irradiated mice were subjected to 2 Gy X-irradiation (RT 305 Philips, The Netherlands, 300 kV, dose rate 0.959 Gy/min). The animals were maintained under permanently controlled microbiological and environmental conditions.

Experimental protocol

The established experimental protocol for IL-1Ra treatment was used (Milenković *et al.* 1995, Jovčić *et al.* 1996ab). Briefly, normal and irradiated mice received two i.p. injections of 50 μ g of rhIL-1Ra (Upjohn, kindly provided by Dr C.A. Dinarello) seventeen and two hours before the animals were sacrificed and tissue and organ cells were harvested. The irradiated mice were sacrificed on day 3 after irradiation. The experiments were performed on 4 to 6 animals per group and were replicated twice.

Cell and plasma harvesting

Whole blood, femurs, spleens, peritoneal exudates and full-thickness skin explants were collected for processing and subsequent cell preparation. Plasma was obtained from heparinized whole blood and frozen at -20°C until assayed for IL-1 levels. For the preparation of bone marrow cell suspensions, the marrow from the femurs was flushed with RPMI-1640 medium (Flow, Irvine, CA, USA). Spleens were gently passed through a wire mash and monodispersed in RPMI-1640 tissue culture medium by passing the cell suspension twice through a 20-gauge needle. Peritoneal exudates were removed by washing the peritoneal cavity with 4-5 ml of saline supplemented with 2 % FCS (Flow, Irvine, CA, USA) and 5 U of heparin/ml. The exudates were immediately centrifuged and the exudate cells collected. Bone marrow, spleen and peritoneal exudate cells were washed once, resuspended in supplemented RPMI-1640 medium and held on ice until cultured.

Cell culture

For the preparation of cell-derived conditioned media (CM), the cells were cultured for 24 h at 37°C in 5 % CO_2 humidified atmosphere in RPMI-1640 supplemented with 5 % FCS and 5×10^{-5} M 2-mercaptoethanol. The cells were plated at a density of $5 \times 10^6/\text{ml}$ (bone marrow or spleen cells) and $2 \times 10^6/\text{ml}$ (peritoneal exudate cells). At the end of the culture period the supernatants were harvested and stored at -20°C until assayed for IL-1 levels.

Preparation of organ-derived conditioned media

Full-thickness skin explants were prepared by a modified procedure described by Rikimaru *et al.* (1991). The explants (1 cm^2) were cultured in small Petri dishes for 24 h at 37°C in serum-free RPMI-1640 medium. Culture medium was harvested, centrifuged and the supernatants collected and stored at -20°C before being assayed for IL-1 levels.

Determination of IL-1 levels

The IL-1 α and IL-1 β were determined using ELISA kits as recommended by the supplier (Genzyme, Cambridge, MA, USA). The sensitivity of these assays enables the detection of cytokine levels as low as 15 pg/ml for IL-1 α and 10 pg/ml for IL-1 β .

Statistical analysis

Data are expressed as means \pm S.E.M. Statistics was performed employing Student's *t*-test.

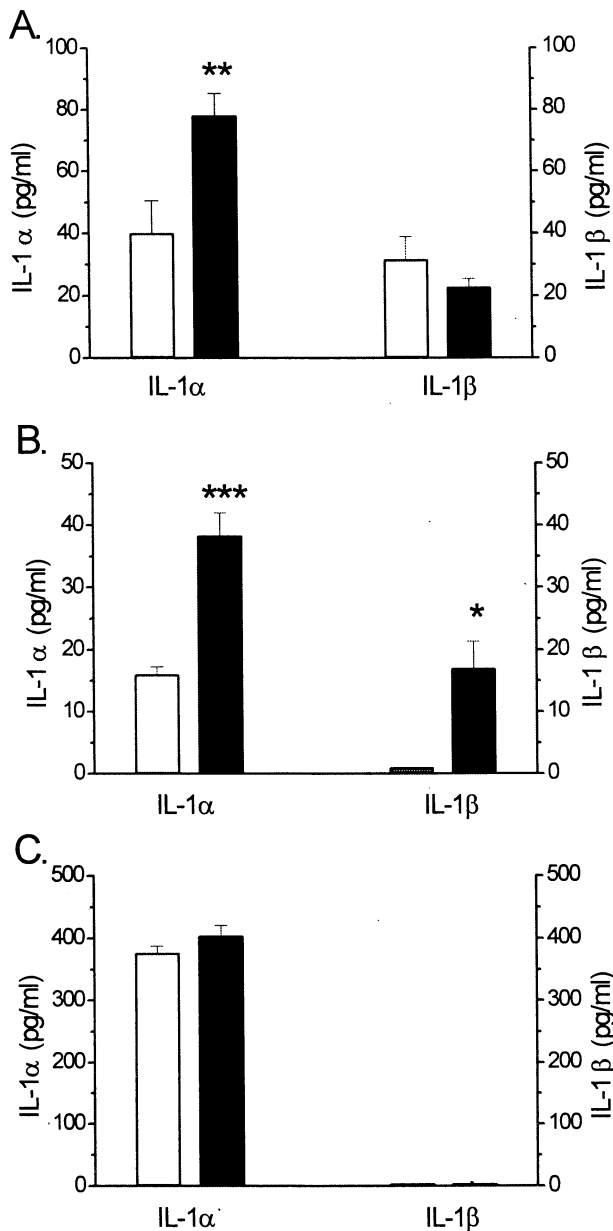


Fig. 1. IL-1 α and IL-1 β levels determined in normal - N (open columns) and sublethally (2 Gy) irradiated CBA mice 3 days after X-ray irradiation - Rx (black columns): A. plasma; B. peritoneal exudate cells-conditioned media; C. full-thickness skin explant-conditioned media. Each data represents mean \pm S.E.M. of values obtained from two experiments. Significant difference compared to normal mice are * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Results

The levels of IL-1 α and IL-1 β were assessed in the plasma and conditioned media (CM) obtained from various cells/organs involved in the hematopoietic, immune and/or inflammatory responses, harvested from normal and irradiated CBA mice, as well as from normal and irradiated mice injected with rhIL-1Ra.

In normal mice, detectable concentrations of IL-1 α and IL-1 β were found in the plasma (Fig. 1A). In peritoneal exudate cells-CM low but detectable basal levels of IL-1 α were observed, whereas IL-1 β concentrations were below detectable level (Fig. 1B). Conditioned media obtained from skin explants contained large amounts of IL-1 α and no detectable levels of IL-1 β (Fig. 1C). In these mice IL-1 α and IL-1 β were below detectable levels in all tested samples of bone marrow cells-CM and spleen cells-CM.

In irradiated mice 3 days after radiation exposure, only the levels of IL-1 α in plasma were significantly elevated above normal values (Fig. 1A). In peritoneal exudate cells-CM, the levels of both IL-1 isoforms were significantly increased as compared to the values obtained in normal mice (Fig. 1B). The conditioned media obtained from postirradiated murine skin explants contained similar large amount of IL-1 α and no detectable levels of IL-1 β as were found in normal mice (Fig. 1C). The concentrations of IL-1 α and IL-1 β in samples of bone marrow cells-CM, as well as of spleen cells-CM obtained from irradiated mice were below detectable levels.

In normal IL-1Ra-treated mice, the levels of both IL-1 isoforms in the plasma were comparable to the values obtained in normal, non-treated mice (Fig. 2A). The rhIL-1Ra-treatment induced a significant elevation of IL-1 β levels in peritoneal exudate cells-CM (Fig. 2B), and IL-1 α in the CM of skin explants (Fig. 2C). In the bone marrow cells-CM, IL-1 α and IL-1 β were below detectable levels. In the samples of spleen cells-CM, after the rhIL-1Ra treatment, the levels of both IL-1 α and IL-1 β were just detectable (12.5 ± 0.3 pg/ml and 8.9 ± 5.4 pg/ml, respectively), i.e. they were increased in comparison to the undetectable levels in normal, non-treated mice.

In irradiated mice treated with rhIL-1Ra, a significant decrease in IL-1 α , and no differences in IL-1 β plasma levels were observed compared to non-treated irradiated mice (Fig. 3A). In peritoneal exudate cells-CM, the IL-1 α concentration was slightly decreased, while the level of IL-1 β significantly

decreased and was below detectable level after rhIL-1Ra treatment (Fig. 3B). Significantly lower amounts of IL-1 α were found in the CM of skin explants obtained from irradiated, rhIL-1Ra-treated mice (Fig. 3C). The

concentrations of IL-1 α and IL-1 β remained below the detection limit in both bone marrow cells-CM and spleen cells-CM of irradiated mice treated with rhIL-1Ra.

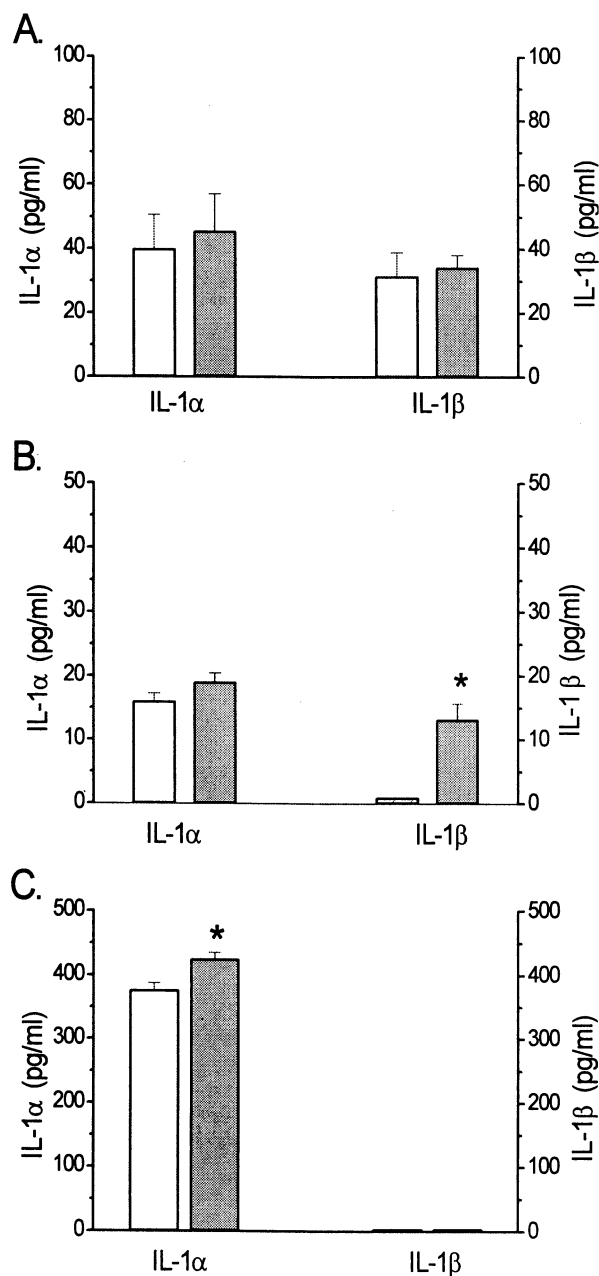


Fig. 2. IL-1 α and IL-1 β levels determined in normal, non-treated (open columns) and IL-1Ra-treated (shaded columns) mice: A. plasma; B. peritoneal exudate cells-conditioned media; C. full-thickness skin explant-conditioned media. Each data represents mean \pm S.E.M. of values obtained from two experiments. Significant difference compared to normal, non-treated mice is $*p < 0.05$.

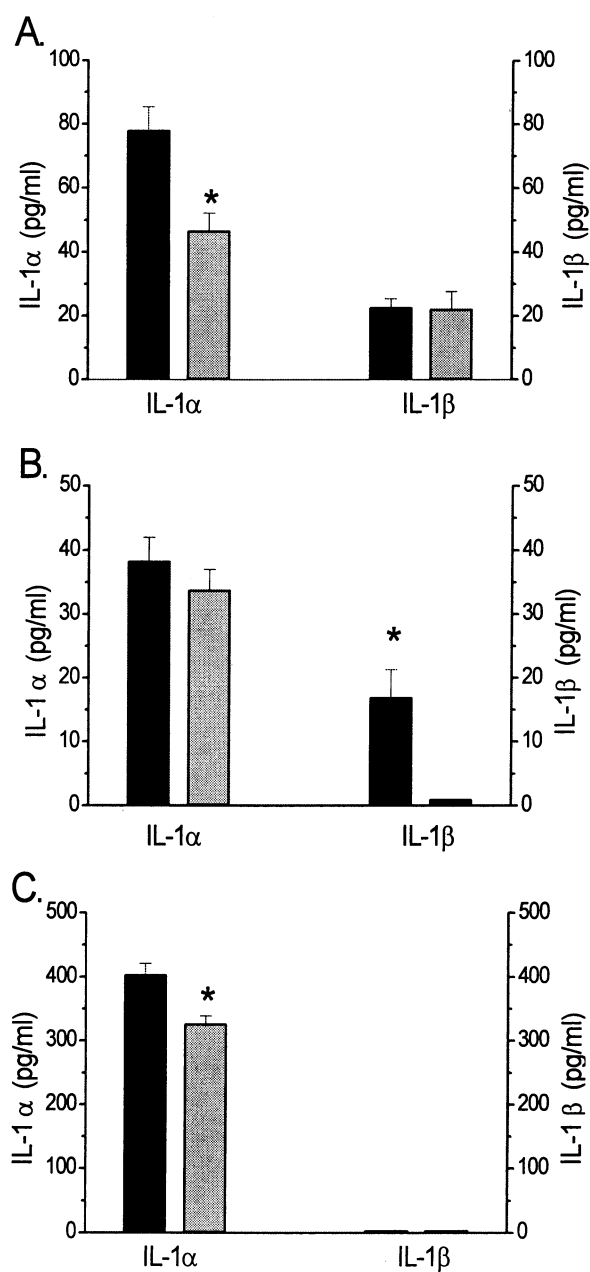


Fig. 3. IL-1 α and IL-1 β levels determined in irradiated, non-treated (black columns) and IL-1Ra-treated (shaded columns) mice: A. plasma; B. peritoneal exudate cells-conditioned media; C. full-thickness skin explant-conditioned media. Each data represents mean \pm S.E.M. of values obtained from two experiments. Significant difference compared to irradiated, non-treated mice is $*p < 0.05$.

Discussion

In our previous studies, where we used rhIL-1Ra to neutralize the endogenous IL-1 activity, we emphasized the importance of the basal levels of IL-1 and IL-1 receptor expression in constitutive, steady-state hematopoiesis, as well as during the recovery of hematopoiesis after irradiation. We suggested that the extent of IL-1 involvement is dependent on the physiological status of the organism (Milenković *et al.* 1995, Jovčić *et al.* 1996ab). In order to clarify the role of IL-1Ra in the regulation of IL-1 production/level, in the present study we examined the effects of *in vivo* rhIL-1Ra treatment on endogenous IL-1 α and IL-1 β levels in CBA mice under conditions associated with different endogenous levels of IL-1, i.e. under steady-state and postirradiation conditions.

In normal, non-treated CBA mice constitutive IL-1 levels were found, implying that IL-1 plays a physiological role in the maintenance of homeostasis. The highest level of IL-1 (IL-1 α) was demonstrated in the skin. It is consistent with various other studies regarding constitutive gene expression and production of IL-1 in cells lining the external environment, i.e. skin and mucosal surfaces (Hauser *et al.* 1986, Rikamuru *et al.* 1991, Dinarello 1994, Matsukawa *et al.* 1997). The observed constitutive production of IL-1 α by peritoneal exudate cells confirmed previous reports that, unlike human cells, murine cells produce and release both IL-1 isoforms in significant amounts (Zuckerman *et al.* 1991, Folliard *et al.* 1992, Torok *et al.* 1995, Dinarello 1996a). However, the finding that IL-1 (both IL-1 α and IL-1 β) circulates in the vascular compartment of normal CBA mice was rather unexpected, since IL-1 is not commonly found in the circulation except during various pathological states (Wakabayashi *et al.* 1991, Cannon *et al.* 1992, Kataranovski *et al.* 1999). In view of current knowledge, IL-1 α is primarily a cytosolic regulator of intracellular events, whereas IL-1 β is a systemic, hormone-like mediator released from cells. However, some naturally occurring conditions that reduce the biological response of IL-1 were reported to interfere with detection of circulating IL-1. Specific, high-avidity autoantibodies directed against IL-1 α , which inhibit the binding of IL-1 α to its receptor (Svenson *et al.* 1990, 1992, Saurat *et al.* 1991), and the binding of IL-1 β to large proteins in the circulation, preferentially soluble type II IL-1 receptors can prevent accurate measurements of IL-1 (Arend *et al.* 1994, Dinarello 1996a). In addition,

recent data (Natea *et al.* 1999) have demonstrated that soluble type I IL-1 receptors can influence the production of IL-1 α , inducing a rapid release of IL-1 α from cells, as well as into the circulation. At present, it is difficult to explain the detection of IL-1 α and IL-1 β in the circulation of CBA mice. We can only speculate that some strain-dependent differences in the expression of these regulatory molecules might be involved. Low or undetectable IL-1 levels were observed in CM obtained from both splenic and bone marrow cell suspensions harvested from normal mice. The presence and the role of IL-1 during constitutive hematopoiesis have been described recently. In rodents, some of the data indicate that the stromal cells from normal mice do not express the IL-1 gene (Barak and Ben-Ishay 1994), while in other studies intramedullary concentrations of IL-1 α were determined (Wang *et al.* 1994). In humans, the ability of normal hematopoietic stem and progenitor cells, obtained from adult bone marrow or umbilical cord blood, to express the IL-1 β gene (Watari *et al.* 1994) as well as the constitutive gene expression of IL-1 β , IL-1Ra and IL-1 β converting enzyme in the bone marrow and peripheral blood of healthy subjects (Cluitmans *et al.* 1995) were reported recently. Our previous results (Jovčić *et al.* 1996b) also provide evidence that IL-1 participates in the regulation of normal steady-state hematopoiesis *in vivo*. Although we were not able to detect significant amounts of released IL-1 in both bone marrow cells and spleen cells-CM, the presence of IL-1 in hematopoietic organs could not be excluded.

In irradiated mice, on day 3 post exposure, significantly increased IL-1 levels were seen in the plasma (IL-1 α) and peritoneal exudate cells-CM (both IL-1 isoforms). There were no significant differences in cytokine protein levels in postirradiation bone marrow, spleen and skin when compared with normal controls. The elevated IL-1 concentrations in the circulation and peritoneal exudate cells-CM confirmed the existence of IL-1 in these compartments of CBA mice and are in accordance with previously reported increase of IL-1 production after irradiation (Dinarello 1996a). Although the undetectable concentrations of IL-1 were found in hematopoietic organs on day 3 post radiation, we have previously pointed out the importance of the endogenous levels of IL-1 in the same experimental model during the recovery of hematopoiesis after sublethal irradiation (Milenković *et al.* 1995, Jovčić *et al.* 1996a). These results are in concordance with the reports of various groups that elevated accumulation of IL-1 α and/or IL-1 β

mRNA, and not always of the protein levels, is found in bone marrow and spleen after irradiation (Baker *et al.* 1995, Ishihara *et al.* 1995, Nemoto *et al.* 1995, Chang *et al.* 1997). However, it is worth to mention that in another experiment (unpublished data) we were able to detect IL-1 α in bone marrow cells-CM on day 1 and in lower amounts on day 2 after the same dose of sublethal irradiation as used here. That implies the time-dependent changes in the *in vivo* IL-1 α protein levels, as were also reported by others (Baker *et al.* 1995).

After the *in vivo* rhIL-1Ra treatment of the animals, were observed significantly elevated IL-1 α and IL-1 β levels in normal mice in the CM obtained from full-thickness mouse skin and peritoneal exudate cells, respectively, and slightly increased IL-1 α and IL-1 β levels in spleen cells-CM. When compared to non-treated mice, no differences were observed in the circulation and bone marrow cells, although the IL-1 levels were still detectable in the circulation and below detectable limits in bone marrow suspensions. These elevated IL-1 levels, obtained after the IL-1 receptors blockade, confirmed our observations that endogenous IL-1 secretion and expression of IL-1 receptors occur as a part of steady-state homeostasis.

On the contrary, the rhIL-1Ra injection in irradiated mice led to decreased concentrations of IL-1 α in the circulation and skin-CM and IL-1 β in peritoneal exudate cells-CM and the unchanged cytokine levels in the culture fluids derived from bone marrow and splenic cells, which were below detection limit. These data suggest that IL-1 production had been reduced. At present, there is little evidence concerning the effects of IL-1Ra on cytokine production *in vivo*. Similar observations that IL-1Ra blocks or reduces the production of IL-1 and other cytokines, which are under the influence of IL-1, have been reported in some animal models of acute or chronic diseases (Dinarello 1996b). Furthermore, decreased levels of several cytokines, including IL-1 β , were found in the circulation of human subjects during experimental endotoxemia when coinjected with IL-1Ra (Granowitz *et al.* 1993). However, it is still not clear what is the impact of the relative concentrations of IL-1 vs. IL-1Ra in health or in the resulting pathological state.

The *in vivo* effects of rhIL-1Ra in normal and irradiated mice pointed out the importance of a balance between IL-1 and IL-1Ra in regulation of IL-1 levels.

Namely, the differential consequences of the same IL-1Ra treatment are related to different endogenous IL-1 levels occurring under steady-state and postirradiation conditions, indicating that the effective concentrations and mutual balance between IL-1 and IL-1Ra are the crucial factors controlling the production of IL-1. While the enhanced IL-1 levels observed in normal mice can be explained by the occupancy of the IL-1 receptors by IL-1Ra, we can only speculate on the mechanisms inducing the decreased IL-1 levels after irradiation. The modulatory effects of IL-1Ra could be ascribed to either suppressed IL-1 production or increased IL-1 receptor expression and hence to increased IL-1 binding. IL-1Ra can act directly or, alternatively, through the other regulatory molecules within cytokine network, which might be activated under its influence. It is well known that postirradiation conditions are associated with increased endogenous secretion of various cytokines and increased sensitivity of cells to the action of regulatory molecules and that cytokines are able to modulate their mutual production. Thus, the IL-1 secretion may be influenced not only by the relative amount of IL-1Ra present, but also by the presence of other cytokines that may supplement or oppose the effects of IL-1Ra.

Our results demonstrating different modulatory effects of IL-1Ra in normal and irradiated mice imply that IL-1Ra itself could be involved in biological reactions induced and enhanced by the irradiation, expressing effects not only related to the IL-1 receptor blockade. Recently, some new, unexpected, non-pathological roles for IL-1Ra in growth, responses to infection and inflammation, and regulation of cytokine expression (Hirsch *et al.* 1996), as well as in health maintenance (Matsukawa *et al.* 1997) have also been reported. Taken together, the evidence currently available has revealed new insights into the function of IL-1Ra, but there are still issues that need to be resolved and should improve our understanding of the physiological/pathological relevance of IL-1Ra.

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Reprint requests

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