Modulation with Cytokines of Radiation Injury: Suggested Mechanisms of Action

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Cytokines, hormone-like proteins, produced by stimulated cells and tissues, were found to protect mice against lethal hematopoietic failure caused by ionizing radiation. Radioprotection was achieved by pretreatment with interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-12, or stem cell factor (SCF) at 18 to 24 hr before irradiation. Pretreatment with antibodies to these cytokines rendered the mice more susceptible to radiation lethality, indicating that these cytokines play a role in innate resistance to radiation. In contrast, treatment with tumor growth factor beta (TGF-β), a cytokine that inhibits cycling of primitive hematopoietic progenitors, sensitized mice to radiation lethality. The schedule of IL-1 administration was critical to its radioprotective effect. Evidence was obtained that this may be based on the induction of additional cytokines by IL-1. The radioprotective effects of cytokines can be based on induction of cycling of primitive progenitor cells (IL-1, SCF), prevention of apoptosis (SCF), and induction of scavenging proteins and enzymes (IL-1, TNF) that reduce oxidative damage. In contrast, radiosensitizing effects may be due to inhibition of progenitor cycling (TGF-β) or enhanced progenitor cell apoptosis (TGF-β). Thus, the insights gained from such studies at the whole-animal level promise a better understanding of the membrane and intracellular events associated with radiation damage and repair of such damage. — Environ Health Perspect 105(Suppl 6):1463-1465 (1997)

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Introduction

Studies in the 1950s indicated that death attributable to hematopoietic damage caused by ionizing radiation can be prevented by pretreatment with immunomodulatory agents (1). Such agents were recognized to stimulate the reticuloendothelial system, but their mechanism of action has not been well defined. The subsequent finding that proinflammatory cytokines can confer radioprotection (2) facilitated a more direct approach to study such mechanisms of protection.

Cytokines produced by stimulated cells and tissues are hormone-like proteins that serve in either autocrine, paracrine, and at times, endocrine fashion, as intercellular messengers. A number of cytokines: interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6 stimulate a broad range of cell and tissue activities (3). These diverse effects depend on the type of signaling receptor and on components of the signal transducing pathway engaged in a given cell, which leads at times to the subsequent activation of different transcription factors. For example, it was recently found that upregulation or the lack of upregulation of the nuclear factor KB (NFKB) lead to a seemingly paradoxical observation — that TNF may lead to apoptosis of some cells yet induce proliferation or differentiation of others (4).

As the characteristics and modes of action of cytokines are becoming rapidly understood, the basis for their protective and/or restorative action is being delineated and should aid in developing a better understanding of the role of inflammatory pathways in producing radiation damage and of protection against such damage. Such understanding should lead to the development of a more optimal treatment aimed at preventing damage to normal tissues. Preclinical and clinical studies demonstrated that a broad range of cytokines can serve to accelerate bone marrow restoration following myeloablative cytotoxic drugs or radiation (5). Although many of these cytokines such as IL-3, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), IL-6, IL-11, erythropoietin (EPO), thrombopoietin (TPO), or stem cell factor (SCF) affect primarily proliferation and differentiation of hematopoietic cells at various stages of their lineage progression, other cytokines such as IL-1, IL-4, or interferon gamma (IFN-γ) are also myelo Restorative. In contrast, only a few of the cytokines, for example IL-1, TNF, SCF, and IL-12, protect animals when given 20 hr before administration of lethal doses of radiation. Several additional cytokines, including TGF-β and IFN-α/β, sensitize to radiation lethality (6).

This paper briefly reviews several of our findings: a) evidence for an important role that IL-1, TNF, IL-6, and SCF play in innate defenses against ionizing radiation; b) evidence that cytokines act in a network, i.e., following cytokine administration to a host, a cascade of additional cytokines and other mediators are induced at different times leading to different outcomes depending on the treatment regimen; and c) evidence that the same cytokine may have opposing effects on different tissues.

Cytokines in Radioprotection and Innate Resistance to Radiation

Administration of cytokines IL-1, TNF, SCF, or IL-12 given within 18 to 24 hr before irradiation protects mice from death. Conversely, anti-IL-1, anti-TNF antibodies, and anti-SCF antibodies in mice not treated with cytokines resulted in LD50/30 doses of radiation becoming lethal to 100% of mice, indicating that the endogenous production of each of these cytokines contributes to innate radiation tolerance (7, 8). Such results provided direct evidence that these cytokines act as endogenous radioprotectors.
Several cytokines were found to synergize in radioprotection. For example, combined administration of IL-1 and TNF or IL-1 and SCF resulted in radioprotection at doses of irradiation at which neither of these cytokines was protective when given separately (8,9). Furthermore, the antibody to the IL-1 receptor blocked TNF-induced radioprotection, whereas the antibody to the TNF blocked IL-1-induced radioprotection. The antibody to SCF blocked IL-1 and TNF induced radioprotection, whereas the antibody to the IL-1 receptor blocked SCF radioprotection. IL-1 also synergized with IL-6, GM-CSF, and G-CSF. However, since IL-1 induces the production of all three of these cytokines, this synergy could be observed only at suboptimal radioprotective doses of IL-1. Thus, the contribution of numerous cytokines to radioprotection seems to be a prerequisite for optimal radioprotection. Each of these cytokines was shown to be upregulated after animal exposure to ionizing radiation (10), providing support for the hypothesis that the therapeutic effects of the supplementary pharmacological doses of these cytokines may be exerted by amplifying the levels of endogenously induced cytokines with the capacity to promote reparative processes.

Cytokine Cascades Determine Protection and Damage from Injury

IL-1 is a potent in vivo stimulator of hemopoiesis partly because of its capacity to induce production of hematopoietic growth factors and to upregulate their receptors on bone marrow progenitors (11). Several hours after administration of a single dose of IL-1 the number of hematopoietic progenitors in the bone marrow increases and reaches a maximum after 48 hr. These early findings suggest that expansion of progenitor cells may be the basis for the myeloprotective action of IL-1. However, since mice receiving irradiation 48 hr after IL-1 treatment are not protected from death (12), the mere increase in the numbers of progenitor cells is not sufficient for IL-1 radioprotection.

The kinetics of the radioprotective effect of IL-1 suggest that this effect may be attributed to the cycling of progenitor cells and to the increased radiodistance of the cells in the late S-phase of the cell cycle (13). Indeed, the administration of IL-1 18 to 24 hr before irradiation that leads to optimal radioprotection coincides with increased sensitivity of progenitor cells to hydroxyurea (HU), selectively toxic for cells in the S-phase. Similarly, pretreatment with IL-1 of mice receiving sublethal doses of 5-fluorouracil (5-FU), a drug that spares early progenitor cells because of their quiescence but is highly toxic to cycling cells, resulted in death of mice and in survival of only 2% of the primitive hematopoietic progenitor cells (12). Death occurred only when IL-1 was given 18 to 24 hr, but not 4 or 48 hr, prior to administration of 5-FU. Apparently IL-1 induced the remaining 98% of these normally 5-FU-resistant, slow-proliferating, or quiescent cells to cycle, perhaps synchronously reaching S-phase at 18 to 24 hr. On the other hand, after 48 hr IL-1 no longer sensitized mice to subsequent sublethal doses of 5-FU and did not affect significantly the numbers of surviving progenitor cells, suggesting that the IL-1-induced cycling effect was transient.

In contrast to radioprotection by pretreatment with a single dose of IL-1 at 18 to 24 hr, two injections of IL-1 48 hr apart, at 72 and 24 hr before irradiation, abrogated radioprotection and the sensitivity of progenitor cells to 5-FU (12). These findings suggest that 48-hr pretreatment with IL-1 results in abrogation of the ability of the early progenitors to cycle in response to subsequent IL-1 challenge. This effect may be based on observed increased expression of TGF-β mRNA and protein (12).

TGF-β has been reported to be a potent inhibitor of the cell cycle for many cell types, acting through the reduction of the expression of multiple growth factor receptors, including c-kit, a receptor for SCF expressed on early hematopoietic progenitors, SCF production, and through activation of cell-cycle inhibitors. TGF-β inhibited the in vitro growth of primitive progenitor cells high proliferative potential colony-forming cells (HPP-CFC) (14).

In addition to TGF-β, other factors induced by IL-1 and inhibitory to cycling and proliferation of hematopoietic progenitors such as prostaglandin and TNF, both of which are induced by IL-1, were reported to inhibit the growth of progenitor cells. Thus, a cascade of cytokines and other mediators induced by IL-1 includes positive as well as negative regulators of cycling of primitive progenitors.

Together these findings suggest that a given cytokine action depends heavily on temporal cascades of cytokines leading to various outcomes at different treatment schedules.

Contrasting Effects of IL-12 on Hematopoietic and Gastrointestinal Tissues

IL-12 has potent antitumor and antimetastatic activity in a number of murine tumor models (15). This property, along with the observation that it is a stimulator of the early hematopoietic progenitor cells, suggest that in addition to playing an important role in cancer therapy IL-12 may act as a myeloprotector. Indeed, administration of IL-12 within 24 hr before otherwise lethal irradiation protected a significant fraction of mice from lethal hematopoietic syndrome (16). The radioprotection was associated with a significant increase in the numbers of hematopoietic progenitor cells found in marrow 3 days after administration of 1200 cGy. IL-12, however, radiosensitized, not radioprotected, the gastrointestinal tract as evidenced in mice that died within 4 to 6 days after receiving IL-12 and 1200 cGy. The gastrointestinal syndrome was documented by gross necropsy and histologic evaluation. Induction of a similar syndrome in mice not treated with IL-12 required doses greater than 1600 cGy. Thus, at doses of radiation at which IL-12 still protects bone marrow cells, it sensitizes the intestinal tract to radiation damage. Although protection of hematopoietic cells was abrogated with anti-IL-1R and anti-SCF antibody, sensitization of the intestinal tract was prevented with anti-IFNγ and anti-TNF antibody (15). Thus, different cytokines are involved in IL-12 protection of the bone marrow than in its sensitizing effect on the gut.

Sensitization of the gut epithelial cells by IL-12-induced IFNγ may be associated with this cytokine's ability to upregulate Fas antigen. This has been demonstrated for lymphocytes and for CD34+ hematopoietic progenitor cells. Although freshly isolated CD34+ cells do not express Fas antigen, stimulation of these cells with IFNγ or TNF-α markedly increased this antigen expression. Ligation of Fas in the presence of IFNγ or TNF-α induces apoptosis of hematopoietic progenitor cells. It is therefore possible that IL-12-induced IFNγ and TNF upregulate gut epithelial cells apoptosis through similar mechanisms, leading to exacerbated gut destruction in irradiated mice. In contrast to the detrimental effect on the gut epithelial cells, IFNγ has been used as a sensitizer in solid tumors.
Conclusions
Complete understanding of how cytokines are radioprotective at the cellular level will only be achieved when we understand how the irradiated cells die. Apoptotic death is presumed to occur in hematopoietic stem cells. There is little direct information about the radiation-induced apoptotic process in these cells because they are rare cells in the host tissues, and culturing them outside the host environment requires conditions that probably are not physiological.

Several possible mechanisms of radioprotection by cytokines have emerged: a) reduction of oxidative damage through induction of mitochondrial enzymes such as MnSOD, and other scavenging proteins, as demonstrated for IL-1, TNF, and IL-6; b) reduction of apoptotic response through induction of bcl-2 by SCF; and c) induction of normally quiescent early progenitor cells to enter the cell cycle and possibly reach the relatively radioresistant late S-phase, as suggested by the kinetics of IL-1 and SCF radioprotection and the susceptibility these cells develop to cycle-dependent drugs HU and 5-FU.

In contrast, the sensitizing mechanisms may include: a) increased oxidative damage that may occur in the absence of scavenger induction, as observed for TNF for many tumor cell line; b) enhanced apoptosis by upregulation of Fas antigen, as reported for TNF and IFNα or activation of hydrolysis of sphingomyelin to ceramide and subsequent downregulation of bcl-2 mRNA expression; and c) arrest of cells in G1 phase of the cycle at the time of exposure to radiation, which may promote apoptosis. Although not completely understood, the growth-arresting effect of TGF-β may be exerted on several molecular targets (14). Among the suggested targets are downregulation of c-myc RNA and protein levels, downregulation of transcriptional induction of cyclin A and cyclin E, and upregulation of inhibitors for cdk-cyclin complexes, p15, p16, p21 and p27. Evidence accumulating from different cell types suggests that there may be substantial differences between different cells in the mechanisms by which TGF-β inhibits their proliferation.

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