CONCISE REPORT

Cytokines in Therapy of Radiation Injury

By Ruth Neta and J.J. Oppenheim

Repeated injections or infusion of hematopoietic growth factors, such as interleukin-3 (IL-3), granulocyte macrophage-colony stimulating factor (GM-CSF), or granulocyte colony stimulating factor (G-CSF), accelerate restoration of hematopoiesis in animals compromised by sublethal doses of cytotoxic drugs or irradiation. Previous work by the investigators has shown that IL-1 induced circulating CSF in normal mice and, when used after sublethal irradiation, accelerated the recovery of endogenous splenic colonies. Therefore, IL-1, as well as IFN-γ, tumor necrosis factor (TNF), G-CSF, and GM-CSF, were evaluated as potential therapeutic agents in irradiated C3H/HeN mice. A single intraperitoneal injection, administered within three hours after a lethal dose (LD100/30) of irradiation that would kill 95% of mice within 30 days, protected in a dose-dependent manner up to 100% of mice from radiation-induced death due to hematopoietic syndrome. Significant therapeutic effects were also achieved with a single dose of IFN-γ or of TNF. In contrast, GM-CSF and G-CSF, administered shortly after irradiation, had no effect in the doses used on mice survival.

A S ATTESTED BY the experience with recent nuclear accidents, there is no effective treatment for patients exposed to doses of radiation that result in fatal hematopoietic failure and/or secondary infections. Clinical difficulties due to HLA mismatching, such as graft v host (GVH) reactions and graft rejection, minimize the successful use of bone marrow transplantation (BMT) in these situations. Therefore, agents that promote repair of bone marrow damage and improve the recovery of the surviving fraction of cells must be identified.

The investigators have reported previously that the cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), but not IL-2, interferon (IFN)-γ, or granulocyte macrophage-colony stimulating factor (GM-CSF), when administered before lethal irradiation, protect mice from death resulting from the hematopoietic syndrome. However, the use of IL-1 following lethal irradiation (lethal dose [LD]100/30) did not affect survival. It has been reported that the hematopoietic growth factors, IL-3, GM-CSF, G-CSF, and CSF-1, accelerate restoration of hematopoiesis in animals compromised by sublethal doses of radiation or by cytotoxic drugs. IL-1 used after irradiation was also effective in accelerating hematopoietic recovery in sublethally (700 cGy) irradiated mice. Furthermore, IL-1 and TNF both induce the appearance of high titers of CSFs in the circulation. The effect of administering a single intraperitoneal dose of IL-1, TNF, GM-CSF, or G-CSF shortly after irradiation of mice has been examined. In addition, IFN-γ was studied to determine if its antiproliferative effect renders it ineffective as a restorative agent.

The investigators present data which show that a single injection of IL-1, in a dose-dependent manner, promotes survival of C3H/HeN mice from a radiation dose that results in death of 95% of control animals within 30 days (LD50/30). IFN-γ had a similar effect, TNF also showed limited therapeutic efficacy, while G-CSF and GM-CSF were not effective in promoting survival.

MATERIALS AND METHODS

Mice. A total of 450 C3H/HeN mice were purchased from Animal Genetics and Production Branch, NCI (Frederick, MD), for use in these experiments. Mice were quarantined on arrival and screened for evidence of disease before being released from quarantine. They were maintained in an AAALAC accredited facility in plastic Micro-isolator cages (Lab Products, Maywood, NY) on hardwood chip contact bedding and provided with commercial rodent chow and acidified tap water (HCI to a pH of 2.5) ad libitum. Animal holding rooms were maintained at 70 ± 2°F with 50 ± 10% relative humidity using at least ten air changes per hour of 100% conditioned fresh air. The mice were on a 12-hour light-dark full spectrum lighting cycle with no twilight. Mice were 8 to 10 weeks of age when used. All cage cleaning, handling, and injections were carried out in a laminar flow clean air unit.

Cytokines. Human recombinant IL-1α was generously provided by Immunex (Seattle) and by Hoffman-La Roche (Nutley, NJ). The preparations were supplied in phosphate buffered saline (PBS) at pH 7.2 or in 30 mmol/L tris-HCl, 400 mmol/L NaCl, pH 7.8, respectively, and used on a weight basis. Human recombinant TNF α, lot number CP4026POS, specific activity 9.6 × 10⁶ U/mg in PBS was a generous gift from Biogen (Cambridge, MA). Murine recombinant GM-CSF was provided by Immunex as a lyophilized powder with sucrose as a stabilizing agent. Human recombinant G-CSF, specific activity 8 × 10⁵ U/mg was a gift from Amgen (Thousand Oaks, CA). Murine recombinant IFN-γ, lot 4296, specific activity 6.8 × 10⁵ U/mg was a gift from Genentech (San Francisco). All reagents were diluted to the desired concentration in pyrogen-free saline just before the single intraperitoneal injection of 0.5 mL to mice, once to three hours after irradiation. All cytokine preparations were assayed for lipopolysaccharide (LPS) contamination in a...
limulus amebocyte assay and determined to contain less than 0.1 ng per inoculum.

Irradiation. Mice were placed in plexiglass containers and were given whole body irradiation at 40 cGy/min by bilaterally positioned 60Co elements. The number of surviving mice was recorded daily for 30 days. Preliminary studies established 800 cGy to be the lethal dose for 95% of mice.

Statistical analysis. Statistical evaluation of the results was done using chi-square analysis.

RESULTS

Effect of IL-1. Doses of IL-1 ranging from 0.1 to 0.5 μg, previously shown to be radioprotective, were administered 20 hours before irradiation.12 In this report a broader range of 0.1 to 5.0 μg doses of IL-1 for therapeutic effects was tested. In six consecutive experiments, a dose-dependent beneficial effect of treatment with IL-1 on survival of 800 cGy irradiated mice was observed (Fig 1A). The most effective doses ranged from 1.0 to 5.0 μg. Up to 100% of mice survived if given a single injection of IL-1 within one to three hours after irradiation with an LD50/30 dose.

Treatment with TNF. Administration of a high dose of 50 μg TNF significantly improved survival (P < .001) (Fig 1B). However, on a weight basis, TNF did not equal the therapeutic potency of IL-1.

Effect of GM-CSF and G-CSF. A single injection of a range of doses of either GM-CSF or G-CSF was administered one to three hours after irradiation. None of these treatments had any effect on mice survival (Fig 1C). This differs from reports indicating that multiple injections of these two growth factors accelerate recovery of the hematopoietic system.4,9

Treatment with IFN-γ. Administration of 1.25 μg of IFN-γ following irradiation of mice with an LD50/30 dose promoted survival (P < .001) (Fig 1D). This result is in contrast to the previous observation that IFN-γ was not radioprotective when used before lethal irradiation.12

DISCUSSION

The results demonstrated that IL-1, TNF, and IFN-γ can promote survival of LD50/30 irradiated mice from radiation-induced death. A single intraperitoneal dose of either cytokine administered one to three hours after irradiation was sufficient to increase mice survival 45% to 100%. Therefore, these data suggest the possibility that IL-1, TNF, and IFN-γ act directly or indirectly to aid in the recovery from radiation damage.

Early studies of damage induced by whole-body exposure to ionizing radiation established the existence of a hematopoietic degenerative phase.13 This phase lasts for several days depending on the species and is characterized by an initial neutrophilia that lasts for several hours and by the appearance of numerous abnormal myelocytic cells first observed in the marrow and several days later in the blood. The elevated numbers of neutrophils in the blood and the elevation in levels of fibrinogen (Neta, unpublished data, 1988), are indicative of an inflammatory reaction to the radiation induced damage. Furthermore, several laboratories previously reported that ionizing radiation causes an increase in phagocytic and bacteriocidical activity of mature neutrophils and macrophages.14,16 The inflammatory response in turn presumably assists in recovery from irradiation by removal of damaged tissues and by promoting the restoration of normal function.

Another characteristic of degenerative phase that develops in parallel with neutrophilia is a temporary mitotic inhibition of the dividing cells, the duration of which is radiation-dose–dependent and lasts for several hours in the LD50/30 dose ranges.13 As dividing cells are the most sensitive to radiation damage, it is the repair of these rapidly dividing cells that may be critical for survival. It is therefore possible that IL-1, TNF, and IFN-γ may change the kinetics of proliferation and recovery and/or promote the repair of damaged cells.

The fact that IL-1 is not effective when used after irradiation in treatment of mice given higher radiation doses than LD50/30 may indicate that a small number of surviving stem cells or progenitor cells may be the necessary targets of IL-1. An additional explanation may depend on the repair of the damaged progenitor cells that may be achieved with IL-1. The reported production of IL-1 following irradiation17,18 suggests that endogenously produced IL-1 may also aid in damage repair.

These studies, as well as studies from other laboratories of effect of cytokines on recovery of damage from radiation or cytotoxic drugs are still preliminary. Given the observed success in improving recovery from radiation damage, the effect of cytokines as adjuncts or substitutes for BMT should be carefully evaluated.
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