Role of Cytokines (Interleukin 1, Tumor Necrosis Factor, and Transforming Growth Factor β) in Natural and Lipopolysaccharide-enhanced Radioresistance

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Summary

Studies of radioresistance and radioprotection provide an excellent in vivo model for dissection of the pathophysiological role of cytokines. The availability of neutralizing antibodies to cytokines has made it possible to assess the contribution of cytokines to host defense and repair processes involved in radioresistance and radioprotection. Administration of anti–interleukin 1 receptor (IL-1R) antibody (35F5) or anti–tumor necrosis factor (TNF) antibody (TN3 19.12) reduced survival of irradiated CD2F1 mice. These results demonstrate conclusively that natural levels of IL-1 and TNF contribute to radioresistance of normal mice. Furthermore, the radioprotective effect of administered IL-1 was blocked not only with anti-IL-1R antibody but also with anti-TNF antibody. Similarly, the radioprotective effect of TNF was reduced with anti-IL-1R antibody. These data suggest that cooperative interaction of both cytokines is necessary to achieve successful radioprotection. Finally, when LPS was used as a radioprotector, the combined administration of anti-IL-1R and anti-TNF not only blocked the radioprotection with LPS, but actually revealed LPS to have a radiosensitizing effect. This effect may be due to induction of TGF-β, since administration of this cytokine results in reduced survival of irradiated mice.

The lethal effects of whole-body exposure to ionizing radiation are due primarily to the destruction of hematopoietic components and subsequent failure of hematopoietic renewal (1, 2). The use of immunostimulatory/inflammatory agents such as LPS before irradiation was shown more than 30 years ago to promote survival from otherwise lethal doses of radiation with subsequent recovery of the hematopoietic system (3, 4). It is now known that many of the pathophysiological in vivo effects of LPS are mediated through the induction of a battery of cytokines, including IL-1 and TNF. We, therefore, hypothesized and established that these two cytokines can confer radioprotection (5, 6). The combined administration of the two cytokines had a synergistic radioprotective effect and was more effective than administration of an optimal dose of LPS (6).

In addition to induction by LPS, IL-1 and TNF are known to be produced in response to stress, infectious agents, and a variety of inflammatory stimuli (7, 8). Most recently, IL-1 and TNF production was shown to be induced after exposure to ionizing radiation (9, 10).

These observations led us to ask several questions. First, do endogenously produced IL-1 and TNF contribute to the enhanced radioresistance of normal mice? Second, do IL-1 and TNF act independently or cooperate in radioprotection? And third, do IL-1 and TNF account for the entire radioprotective effect of LPS? The results reported here provide evidence that IL-1 and TNF are essential for natural as well as for immunomodulator-enhanced radioresistance.

Materials and Methods

Mice. CD2F1 male and C3H/HeN female mice were purchased from the Animal Genetics and Production Branch, National Cancer Institute (Frederick, MD). C3H/HeJ female mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were handled as previously described (6).

Cytokines. Recombinant human IL-1α (rhIL-1α)[1] (117-271 Ro 24-5008; lot IL-1 2/88; sp act 3 × 10⁸ U/mg) was kindly provided by Dr. Peter Lomedico, Hoffmann-La Roche Inc. (Nutley, NJ). Recombinant human TNF-α (rhTNF-α) (lot CP4026P08 in PBS; sp act 9.6 × 10⁶ U/mg protein) was obtained from Biogen (Cambridge, MA), and recombinant murine TNF-α (rmTNF-α) (lot 4296-17; sp act 2 × 10⁸ U/mg, as assayed on L929 cells in our laboratory) was kindly provided by Genentech (San Francisco, CA). TGF-β (lot 8987-53) was kindly provided by Dr. Palladino of Genentech. LPS (protein-free prepared from *Escherichia coli* K235 by the phenol-water extraction method) was kindly provided by Dr. Stefanie Vogel of Uniformed Services University for the Health Sciences (Bethesda, MD).

Antibodies. Anti-IL-1R antibody, mAb 35F5, was raised in rats against cloned IL-1R isolated from EL 4 cells as previously described (11). Anti-murine TNF antibody, mAb TN3.19.12, was raised in Armenian hamsters against murine TNF-α as previously described (12). Rat Ig (Sigma Chemical Co., St. Louis, MO) was used as a control for 35F5 antibody. As controls for TN3.19.12, anti-murine IFN-γ antibody (H22, an antibody raised in hamsters against murine IFN-γ) or hamster IgG (L2.3D9 raised in Armenian hamster against human IL-2, as described [12]) were used.

The antibodies and recombinant cytokines were diluted in pyrogen-free saline on the day of injection. The antibodies were given intraperitoneally 6–20 h before intraperitoneal injection of the cytokines.

Irradiation. Mice were randomized, 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 d.

*Statistical Analysis.* Statistical evaluation of the results was carried out using χ^2 analysis and Cox Mantel test.

Results

The Role of IL-1 and TNF in Natural Radioresistance. To test whether endogenously produced IL-1 and TNF contribute to the natural radioresistance of normal mice, mice were treated with anti-IL-1R antibody, which blocks the activities of both IL-1α and IL-1β (11), or with anti-TNF antibody (12). Administration of anti-IL-1R antibody (35F5) or anti-TNF antibody (TN3 19.12) to LD40/30 irradiated mice reduced their survival (Fig. 1). The apparent radiosensitizing effect observed in normal mice given either antibody 20 h before irradiation was equal to that observed in mice given the antibody 1 h after irradiation. Thus, these antibodies exacerbate radiation damage by blocking the beneficial effects of cytokines even when produced after the completion of radiation exposure.

Do IL-1 and TNF Act Independently or Cooperate in Radioprotection? IL-1 and TNF are well documented to induce one another in vitro (13–16). We examined the interdependence of IL-1 and TNF in radioprotection by testing the effect of neutralization of TNF in IL-1-injected mice and receptor blockage of IL-1 in TNF-injected mice (Fig. 2). Anti-IL-1R antibody, given to mice before TNF administration, reduced the proportion of TNF-radioprotected mice from 60% to 15%. Similarly, anti-TNF antibody reduced IL-1-induced radioprotection from 88% to 40%. These in vivo results show

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[1] *Abbreviations used in this paper:* rh, recombinant human; rm, recombinant murine.
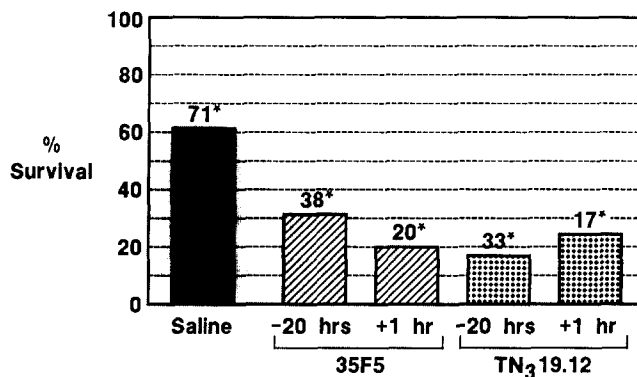
Figure 1. Radiosensitizing effect of anti-IL-1R antibody (35F5) and anti-TNF antibody (TN3 19.12.). CD2F1 male mice 8–10 wk old received intraperitoneal injections of 100 μg 35F5, 20 h before or 1 h after irradiation with 825 cGy ⁶⁰Co. TN3 19.12 antibody was given at 100 μg 20 h before irradiation or at 50 μg 1 h after irradiation. Groups of control mice were given 100 μg of rat IgG, 100 μg anti-murine IFN-γ, or 100 μg of hamster IgG, or vehicle-saline injection of 0.5 ml per mouse. The survival of mice given 825 cGy was recorded daily for 30 days. The survival time of control mice receiving anti-IFN-γ, rat IgG, or hamster IgG did not differ from mice given saline injections (at 66, 50, and 68%, respectively; not shown in the figure). The survival of mice receiving 35F5 or TN3 19.12 before or after irradiation was significantly different from that of control mice (p < 0.01). (*) The number of mice used in the experiments.

that TNF contributes to optimal radioprotection with IL-1, and that IL-1 participates in radioprotection with TNF.

Do IL-1 and TNF Account for the Entire Radioprotective Effect of LPS? The relative contribution of IL-1 and TNF in LPS-
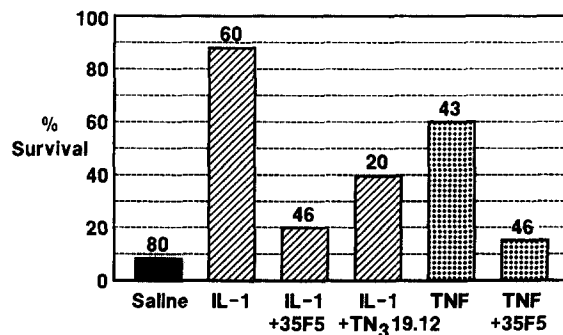
Figure 2. Anti-IL-1R antibody (35F5) reduces TNF-induced radioprotection, and anti-TNF antibody (TN3 19.12) reduces IL-1-induced radioprotection. CD2F1 mice received intraperitoneally 100 μg of 35F5 or 200 μg of TN3 19.12. Groups of control mice received injections of equivalent amounts of rat IgG or hamster IgG (L2.3D9) or saline injections. 20 h later, groups of mice received either 300 ng rhIL-1, 5 μg of rhTNF-α, or 1 μg of rmTNFα. After an additional 20 h, mice received 950 cGy of ⁶⁰Co radiation. The survival time was recorded for 30 d. The survival of mice receiving rhTNF or rmTNF was similar and the groups were therefore combined. Control mice receiving rat IgG or hamster IgG and IL-1 did not differ significantly in survival from IL-1 only-treated mice (at 80 and 95%, respectively). 65% of mice given hamster IgG before TNF survived. The numbers at the top of the bars indicate the total number of mice used in eight experiments. The survival of mice receiving 35F5 and IL-1 or TN3 19.12 and IL-1 differed significantly (p < 0.01) from survival of mice receiving IL-1 alone. Similarly, the survival of mice receiving 35F5 and TNF differed significantly (p < 0.01) from that of mice receiving TNF alone.

induced radioprotection was investigated using neutralizing antibodies. Anti-IL-1R antibody given to mice before LPS and lethal irradiation reduced survival from 74% to 20% (Table 1). Similarly, anti-TNF antibody given before LPS reduced survival to 30%. Combined administration of these antibodies completely abolished the radioprotective effect of LPS, demonstrating that this effect is due entirely to the combined effect of IL-1 and TNF. Furthermore, administration of both antibodies actually led to a reduced survival time of LPS-treated mice to less than that of nontreated mice (Figure 3). Thus, inhibition of IL-1- and TNF-mediated effects of LPS revealed LPS to have latent radiosensitizing capabilities.
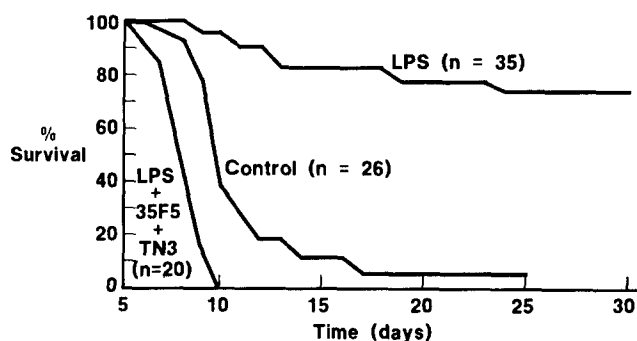
The Effect of TGF-β on Survival of Irradiated Mice. Based on the above observations, we asked whether some of the cytokines induced by LPS may contribute to increased radiation lethality. LPS induces secretion of TGF-β from human blood monocytes (17). We tested the radiosensitizing effect of TGF-β because of its previously observed activity of inhibiting proliferation of early bone marrow progenitor cells, and of inhibiting many of the biologic effects of IL-1 and TNF (18-22). Indeed, TGF-β given to mice either before or after lethal irradiation resulted in a dose-dependent reduction in survival (Fig. 4).

TGF-β was also radiosensitizing for C3H/HeJ and C3H/HeN mice. 85% (17/20) of control C3H/HeN mice survived 725 cGy radiation, whereas only 10% (1/10) of mice given 10 μg TGF-β 16 h before, and 15% (3/20) given TGF-β 1 h after irradiation, survived. Similarly, none of the C3H/HeJ mice given 10 μg TGF-β 1 h after irradiation with an $LD_{50/30}$ survived. Thus, the sensitizing effect of TGF-β to radiation lethality is not restricted to a particular mouse strain. Combined administration of IL-1 and TGF-β, however, did
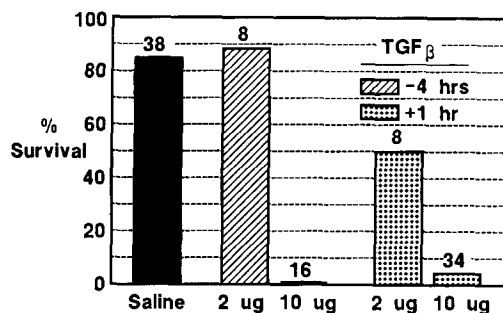

Figure 3. Effect of anti-IL-1R antibody and anti-TNF antibody on LPS-induced radioprotection. Groups of mice received treatment as described in Table 1. The daily record of survival of mice given a combination of 200 μg of 35F5 and 200 μg of TN3 19.12 antibody and LPS, LPS alone, or saline control and 950 cGy irradiation is presented. The mean survival time of LPS- and antibody-treated mice was significantly reduced as compared with the control mice ($p < 0.001$ using Cox Mantel test).

not result in any reduction of the radioprotective effect of IL-1 (results not shown), indicating that the presence of IL-1 masks the radiosensitizing effects of TGF-β.

Discussion

Cytokines have been shown to have so many complex effects that it has become virtually impossible to predict their relevant role from the in vitro models. Consequently, it is more imperative than ever to evaluate their pathophysiological role in vivo. Studies of the role of cytokines in counteracting the lethal effects of radiation provide a useful model for evaluating the in vivo role of these cytokines in promoting the restoration of hematopoiesis and consequent enhancement of host resistance to infections. Our results showing that greater number of mice receiving anti-IL-1R antibody or anti-TNF antibody die after exposure to ionizing radiation indicate that endogenously produced IL-1 and TNF play an important role in the host's ability to recover from lethal radiation. The specificity of anti-IL-1R antibody was previously demonstrated

Table 1. *Effect of Anti-IL-1 Antibody and Anti-TNF Antibody on LPS-induced Radioprotection*

Group	Treatment LPS	Treatment Antibody	Dead/ total	Percent survival
1	–	Saline	56/59	5
2	+	Saline	16/61	74
3	+	Rat IgG	12/36	67
4	+	Hamster IgG	3/18	83
5	+	Anti-IL-1R	24/30	20
6	+	Anti-TNF	14/20	30
7	+	Anti-IL-1R – anti-TNF	20/20	0

CD2F1 mice received intraperitoneally 200 μg anti-IL-1R, 200 μg anti-TNF, or both. Groups of control mice received 200 μg hamster IgG, rat IgG, or saline injections. 20 h later, mice received 1 μg E. Coli LPS intraperitoneally, and 1 d later were given 950 cGy gamma radiation. The survival time was recorded for 30 d. The survival of mice in groups 5 and 6 was significantly reduced ($p < 0.01$) compared with survival of mice in groups 2, 3, and 4. There was no significant difference in the survival of groups 2, 3, and 4.

Figure 4. Radiosensitizing effect of TGF-β. CD2F1 mice received intraperitoneal injections of rHTGF-β at times before and after irradiation in quantities as indicated, or saline injection. The results are a summary of three experiments. The survival of mice receiving 10 μg TGF-β before or after irradiation differ significantly ($p < 0.001$) from that of saline-treated mice.

since passive immunization of mice with 35F5 antibody reduced IL-1-induced radioprotection, and also reduced IL-1-induced serum levels of IL-6, CSF, and serum amyloid P (23, 24). Similarly, administration of anti-TNF antibody, TN3-19.12, to mice subsequently treated with LPS specifically prevented the appearance of TNF in the circulation (12). Serum levels of 35F5 and TN3 19.12 were previously shown to plateau within 6–8 h and to remain elevated for 2–7 d after injection (11, 12). Thus, the data showing that administration of antibody after irradiation has an effect similar to antibody given before irradiation suggest that radiation-induced IL-1 and TNF confer radioresistance by promoting repair and restoring host defenses after lethal radiation damage. This effect of endogenously produced IL-1 and TNF is augmented by administration of an exogenous supply of these cytokines, as previously observed (5, 6, 25).

Our observation that anti-IL-1R antibody largely blocks TNF-induced radioprotection whereas anti-TNF antibody partially blocks IL-1-induced radioprotection suggests that mutual induction of IL-1 by TNF and TNF by IL-1 occurs in vivo as well as in vitro (13–16), and their subsequent interaction is necessary to achieve optimal radioprotection. This is in agreement with a report by Dinarello et al. (26) demonstrating that the second pyrogenic phase induced by supernatants from TNF-stimulated mononuclear cells could be neutralized by anti-IL-1 antibody. We have also observed that the levels of CSF induced in circulation after challenge with IL-1 can be reduced by anti-TNF antibody, whereas TNF-induced CSF is reduced by anti-IL-1R antibody (Neta et al., unpublished results). Consequently, mutual induction of TNF and IL-1 may be required not only for achieving in vivo radioprotection, but also for other in vivo effects. The generally observed overlapping activities of IL-1 and TNF (27, 28) may reflect a cellular requirement for triggering by both signals, and administration of either IL-1 or TNF alone would result in the production of the necessary second signal; thus accounting for their apparent ability to act by themselves.

LPS is known to induce a battery of cytokines, including IL-1, TNF, CSF, IL-6, IL-8, IFN, and TGF-β (29–33). We have previously shown that granulocyte CSF, granulocyte/macrophage CSF, and IL-6, when administered together with suboptimal doses of IL-1, synergize in radioprotection (6, 36). However, the present results indicate that neutralization of TNF and IL-1 not only completely blocks the radioprotective effect of LPS but also unexpectedly reveals LPS to have radiosensitizing properties. Since administration of TGF-β results in greatly enhanced mortality of irradiated mice, and LPS is a known inducer of TGF-β, we assume that in the absence of IL-1 and TNF, other LPS-induced cytokines, IL-6, granulocyte CSF, or granulocyte/macrophage CSF, cannot prevent radiation induced lethality. The finding that when given with IL-1, TGF-β does not reduce IL-1-induced radioprotection, provides further support that IL-1 is critical for radioprotection. Whether the enhanced lethality observed due to TGF-β is the direct result of: (a) inhibiting proliferation of early progenitor cells; (b) impairment of IL-1 and TNF synthesis, as previously shown (34); or (c) reduction of IL-1R expression (35) remains to be established. It is possible that other cytokines induced with LPS, in addition to TGF-β, may also contribute to radiosensitization. Indeed, we have previously shown that IL-6 given alone before irradiation acts also as a radiosensitizer (36).

In conclusion, the present results provide the first demonstration that: (a) the endogenously produced cytokines, IL-1 and TNF, contribute to natural radioresistance; (b) IL-1 and TNF, even when given separately, interact with one another to produce radioprotection; (c) radioprotection with LPS depends on induction of IL-1 and TNF, since blocking the activities of these two cytokines completely abolishes the radioprotective effect of LPS; (d) in the absence of IL-1 and TNF, LPS exerts a radiosensitizing effect; and (e) TGF-β renders mice more sensitive to radiation. Thus, LPS induces a mixture of radioprotective and radiosensitizing cytokines. These cytokines also mediate the immunomodulating effects of LPS. Consequently, therapeutic use of selected cytokines may be preferable to the use of exogenous immunomodulators such as LPS, which induce mixtures of cytokines capable of counteracting each other's effects.

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