Regulation of Tumor Necrosis Factor Gene Expression by Ionizing Radiation in Human Myeloid Leukemia Cells and Peripheral Blood Monocytes

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Abstract

Previous studies have demonstrated that ionizing radiation induces the expression of certain cytokines, such as TNF α /cachectin. However, there is presently no available information regarding the molecular mechanisms responsible for the regulation of cytokine gene expression by ionizing radiation. In this report, we describe the regulation of the TNF gene by ionizing radiation in human myeloid leukemia cells. The increase in TNF transcripts by x rays was both time- and dose-dependent as determined by Northern blot analysis. Similar findings were obtained in human peripheral blood monocytes. Transcriptional run-on analyses have demonstrated that ionizing radiation stimulates the rate of TNF gene transcription. Furthermore, induction of TNF mRNA was increased in the absence of protein synthesis. In contrast, ionizing radiation had little effect on the half-life of TNF transcripts. These findings indicate that the increase in TNF mRNA observed after irradiation is regulated by transcriptional mechanisms and suggest that production of this cytokine by myeloid cells may play a role in the pathophysiologic effects of ionizing radiation. (*J. Clin. Invest.* 1991; 87:1794-1797.) Key words: ionizing radiation • TNF • gene expression • transcription • monocytes

Introduction

TNF α /cachectin is a 17-kD polypeptide with pleiotropic effects (1). TNF induces procoagulant activity both in vitro and in vivo (2, 3); this response may be responsible for the TNF-induced hemorrhagic necrosis of heterotransplanted human tumors (4). TNF also has direct cytostatic and cytotoxic effects on transformed cells in culture (5-7). The demonstration that TNF is identical to cachectin (8) has implicated this factor in the inhibition of lipoprotein lipase activity and de novo synthesis of triglycerides (9). TNF is a pivotal mediator of endotoxic shock and end-organ tissue damage (10). The pathogenesis of a number of diseases including cerebral malaria, graft-vs.-host disease, and pulmonary fibrosis has also been associated with release of TNF (11-14). Moreover, TNF is induced by a number of chemical and biologic stimuli. For example, LPS, phorbol esters, viruses, complement components, and certain cyto-

kines, such as IL-1 and TNF itself, have been shown to induce TNF gene expression (reviewed in reference 15).

Ionizing radiation is responsible for the induction of neoplastic transformation as well as cell killing. However, the cellular and molecular mechanisms involved in mediating these effects of ionizing radiation are not well understood. Recent studies have demonstrated that radiation treatment is associated with increased expression of certain growth factors and cytokines. For example, platelet-derived growth factor and fibroblast growth factor are released from endothelial cells treated with x rays (16), while interleukin 1 mRNA levels are increased after irradiation of Syrian hamster embryo cells (17). We have recently demonstrated that levels of TNF mRNA and protein are increased in irradiated human sarcoma tumor cultures (18). Furthermore, the addition of exogenous sublethal concentrations of TNF enhances cell killing by radiation in sarcoma tumor cell lines (18). These studies suggest that certain cytokines, such as TNF, may play a role in radiation-induced injury and/or transformation. However, there are presently no available insights regarding the mechanisms responsible for the induction of gene expression by radiation.

In these studies, we have investigated the effects of ionizing radiation on the regulation of TNF gene expression. The results show that ionizing radiation regulates TNF transcripts in human myeloid leukemia cells as well as in human peripheral blood monocytes in a time- and dose-dependent manner. Furthermore, the results also demonstrate that ionizing radiation regulates TNF gene expression by a transcriptional mechanism.

Methods

Cell culture. HL-60 and U-937 cells (American Type Culture Collection, Rockville, MD) were grown as described (19). Peripheral blood monocytes were isolated and cultured as described (20). Cells were irradiated at room temperature using a Gammacell 1000 (Atomic Energy of Canada Ltd., Ontario) with a ^{137}Cs source emitting at a fixed dose rate of 14.3 Gray (Gy)/min as determined by dosimetry. Control cells were exposed to the same conditions but not irradiated.

Northern blot analysis. Total cellular RNA was isolated by the guanidine isothiocyanate/cesium chloride method (21), separated in a 1% agarose/2.2 M formaldehyde gel, transferred to a nitrocellulose filter (Schleicher & Schuell, Inc., Keene, NH), and hybridized to the following ^{32}P -labeled DNA probes: (a) the 1.9-kb BamHI/PstI human TNF cDNA purified from the pE4 plasmid (22), and (b) the 2.0-kb PstI β -actin cDNA purified from the pA1 plasmid (23).

Run-on transcriptional analysis. HL-60 cells were treated with ionizing radiation and nuclei were isolated as described (19). Newly elongated nuclear RNA was labeled with [^{32}P]UTP as described (19) and hybridized to the following plasmid DNAs: (a) the 1.9-kb BamHI/PstI insert of the human TNF cDNA (22); (b) the 1.1-kb BamHI insert of the human β -globin gene (negative control) (24); and (c) the 2.0-kb PstI insert of the chicken β -actin gene (positive control) (23). The digested

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DNAs were run in 1% agarose/TAE (TAE: 40 mM Tris-acetate, pH 8.0, 2 mM Na₂EDTA), transferred to nitrocellulose filters, and hybridized as previously described (19). Autoradiographic bands were scanned using an LKB Produkter (Bromma, Sweden) Ultrosan XL laser densitometer and analyzed with the Gelscan XL software package (version 1.21).

Results and Discussion

TNF expression is induced by ionizing radiation in myeloid cell lines. The induction of TNF by irradiation was first suggested when medium removed from irradiated cultures of human sarcoma cell lines was cytotoxic to these as well as other tumor cell lines. We subsequently demonstrated that the level of TNF mRNA and protein is increased after treatment with x rays in certain human sarcoma cells (18). However, the molecular basis for this effect has remained unclear. Although human epithelial cells produce TNF (25), hematopoietic cells such as monocytes and macrophages are the major source of TNF production and release. Consequently, in this study, we first examined the effects of ionizing radiation on TNF gene expression in human HL-60 promyelocytic leukemia cells. Low levels of TNF transcripts were detectable in untreated HL-60 cells and increased by 1 h after treatment with ionizing radiation (Fig. 1 A). A maximal 8.1-fold increase in levels of TNF mRNA was detected after 3 h (Fig. 1 A). In contrast, there was little change in the level of actin transcripts following treatment of HL-60 cells with ionizing radiation (Fig. 1 A).

Figure 1. Effects of ionizing radiation on TNF expression in HL-60 myeloid leukemia cells. (A) HL-60 cells were exposed to 20 Gy ionizing radiation. Total cellular RNA (20 µg) was isolated at the indicated times and hybridized to a ³²P-labeled TNF or actin probe. (B) HL-60 cells were exposed to varying doses of irradiation and RNA collected after 3 h as described. Lanes labeled HL-60 indicate untreated cells.

The effects of varying doses of ionizing radiation were also examined in these cells. Irradiation with doses as low as 2 Gy was associated with detectable increases in the level of TNF transcripts (Fig. 1 B). The induction of TNF was maximal after exposure to 50 Gy ionizing radiation (Fig. 1 B). Taken together, these findings suggested that the increase in TNF mRNA after exposure to x rays is both time and dose dependent.

The effects of ionizing radiation on TNF gene expression were also examined in other myeloid cells. For example, human U-937 monocytic leukemia cells similarly had low but detectable levels of TNF transcripts. Irradiation of these cells was associated with a time-dependent increase in the level of TNF mRNA (Fig. 2 A). The level of these transcripts was maximal by 1 h (3.9-fold increase) and returned to control levels by 6 h after ionizing radiation treatment (Fig. 2 A). In view of these findings in myeloid leukemia cells, it was of interest to examine the effects of ionizing radiation on TNF expression in human monocytes. TNF mRNA was at low levels in resting monocytes (Fig. 2 B). However, treatment of these cells with ionizing radiation resulted in an increase in TNF gene expression by 1 h (Fig. 2 B). These findings suggested that TNF expression is regulated by x rays in both myeloid cell lines and normal monocytes.

TNF gene expression is regulated at the transcriptional level by ionizing radiation. Previous studies have investigated the mechanisms of TNF gene regulation in mouse macrophages. TNF is actively transcribed in thioglycollate-elicited peritoneal macrophages, while an increase in the rate of TNF transcription was observed after treatment with LPS or interferon-γ (26, 27). We have previously demonstrated that TNF induction by 12-tetradecanoylphorbol-13-acetate (TPA)¹ is regulated by transcriptional mechanisms in human peripheral blood monocytes as well as in myeloid leukemia cell lines (20, 28). In order to determine the level of regulation of TNF gene expression by ionizing radiation, we first measured the rate of TNF transcription in isolated nuclei using the run-on transcription assay method. As shown in Fig. 3, the actin gene was actively transcribed and the β-globin gene was transcriptionally inactive in both untreated and irradiated HL-60 cells. Low levels of TNF gene transcription were also detectable in untreated cells. However, ionizing radiation treatment was associated with an increase in TNF gene transcription after 3 h of exposure (Fig. 3).

The effects of protein synthesis inhibition on TNF gene expression were also examined in HL-60 cells treated with ionizing radiation. Cycloheximide treatment alone was associated by a low but detectable increase in the level of TNF transcripts (Fig. 4 A). Exposure of cells to ionizing radiation and cycloheximide increased levels of TNF mRNA by 3.0-fold as compared with treatment with ionizing radiation alone (Fig. 4 A). These changes in TNF gene expression were associated with little effect on actin mRNA levels (Fig. 4 A). In contrast, cycloheximide had no effect on the rates of TNF transcription as monitored by nuclear run-on assays (data not shown). In this regard, cycloheximide may inhibit the synthesis of a labile protein that affects the turnover of TNF mRNA (20). These results may be related to the presence of a conserved AU sequence present in the 3' untranslated region of the TNF mRNA (29), which may comprise a posttranscriptional regulatory element involved in

1. Abbreviations used in this paper: TPA, 12-tetradecanoylphorbol-13-acetate.

Figure 2. Effects of ionizing radiation on TNF mRNA levels in other myeloid leukemia cells and human monocytes. (A) U-937 cells were treated with 20 Gy ionizing radiation and RNA (20 μ g) was isolated at the indicated times. (B) Normal human peripheral blood monocytes were treated with 20 Gy ionizing radiation for the indicated times. Total cellular RNA (15 μ g) was hybridized to the ^{32} P-labeled TNF cDNA probe. Lanes labeled U-937 and monocytes indicate untreated cells.

control of TNF gene expression. These findings also suggest that induction of TNF mRNA by ionizing radiation is independent of new protein synthesis and that transcription factors regulating expression of the TNF gene are already present within the cell.

In order to study the posttranscriptional regulation of TNF mRNA by ionizing radiation, the stability of TNF transcripts was determined in both untreated and x-ray treated HL-60 cells. Untreated HL-60 cells exposed to actinomycin D for varying times resulted in a decrease in the level of constitutively expressed TNF transcripts (Fig. 4 B). The half-life of TNF mRNA as determined by densitometric scanning was 20 min. HL-60 cells were also treated with 20 Gy ionizing radiation to induce TNF expression, and then exposed to actinomycin D to inhibit further transcription. As shown in Fig. 4 B, the half-life of TNF mRNA after irradiation was determined to be 20 min. Taken together, these results suggest that ionizing radiation has little, if any, effect on the stability of TNF transcripts. Moreover, these results and others, (20) demonstrating the relatively short half-life of TNF mRNA, support the findings that increased transcription of the TNF gene is coincident with the

Figure 3. Effects of ionizing radiation on the transcriptional rate of TNF gene expression. HL-60 cells were treated with 20 Gy ionizing radiation. After 3 h, nuclei were collected and ^{32} P-labeled RNA was isolated as described in Methods. Hybridizations were performed using 1×10^7 cpm/ml

and actin ($\square$), β -globin ($\blacksquare$), or TNF ($\blacksquare$) DNA immobilized on a nitrocellulose filter. Autoradiographs were scanned by laser densitometry. The intensity of the actin band was normalized for both control and irradiated (XRT) cells. Results represent mean $\pm$ SD of two independent experiments.

Figure 4. Effects of cycloheximide or actinomycin D on the induction of TNF gene expression by ionizing radiation. (A) HL-60 cells were treated with 20 Gy ionizing radiation in the presence or absence of 5 μ g/ml cycloheximide (CHX). Total cellular RNA (20 μ g) was isolated at the indicated times and hybridized to a ^{32} P-labeled TNF or actin cDNA probe. (B) Actinomycin D (Act) (5 μ g/ml) was added to untreated HL-60 cells or to HL-60 cells 3 h after exposure to 20 Gy ionizing radiation. RNA (20 μ g) was isolated at the indicated times (after the addition of the actinomycin D) and hybridized to a ^{32} P-labeled TNF probe.

maximal steady-state levels observed by Northern blot analysis.

The 5' flanking promoter region of the human TNF gene has been recently studied in a transient transfection system using the U-937 cell line (30). A cyclic AMP responsive element, as well as AP-1 and AP-2 motifs, have been described in the 5' sequence of the human TNF gene (30). Induction of the TNF gene by TPA has been localized to a 36-bp sequence immediately upstream from the transcription start site (30). The polypeptide product of the *c-jun* gene is a component of the AP-1 transcription factor complex. In this regard, we have recently shown that ionizing radiation regulates *c-jun* gene expression in human myeloid leukemia cells, as well as diploid fibroblasts (31). Treatment of these cells with ionizing radiation also increases expression of other members of the AP-1 complex including *jun-B* and *c-fos* (31). However, it is not clear whether AP-1 plays a role in the control of TNF gene transcription. In this regard, recent data suggest that NF- κ B motifs play a key role in the LPS-mediated transcriptional activation of the TNF gene (32, 33). Additional studies are now needed to define the *cis*-acting elements in the TNF promoter which are responsible for the regulation of TNF gene expression by ionizing radiation.

Although little is known about the intracellular signal transduction pathways activated by ionizing radiation, recent findings suggest the involvement of protein kinase C. In this regard, transcriptional activation of a construct containing the long terminal repeat of Moloney murine sarcoma virus linked to the chloramphenicol acetyltransferase gene was examined after x irradiation (34). Exposure to ionizing radiation stimulated expression twofold, while treatment with H7, a nonspecific isoquinolinosulfonamide inhibitor of protein kinases, totally abrogated the response to x rays (34). Furthermore, preincubation with TPA to downregulate protein kinase C also abolished the response of x rays (34). Together with the results demonstrating the induction of TNF gene expression by phorbol esters (20, 28), these findings suggest that the effects of ionizing radiation on TNF gene expression might also be mediated by a protein kinase C-dependent mechanism.

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