Effects of GM-CSF, IL-3, and GM-CSF/IL-3 fusion protein on apoptosis of human myeloid leukemic cell line Tf-1 induced by irradiation

Su-rong YANG, Li WEN, Ying-qing LU, Qin-yan GONG, Rong YU, Ming-hui YAO[1]

Department of Pharmacology, Shanghai Medical College, Fudan University, Shanghai 200032, China

KEY WORDS granulocyte-macrophage colony-stimulating factor; interleukin-3; recombinant fusion proteins; cultured cells; radiation; apoptosis; caspases; flow cytometry

ABSTRACT

AIM: To observe the effects of three cytokines on the apoptosis of Tf-1 cells induced by γ irradiation and investigate the relationship between apoptosis and caspase-3 activity. **METHODS:** Different cytokines GM-CSF, IL-3 and GM-CS/IL-3 fusion protein were added into the irradiated Tf-1 cells. MTT assay, morphology, flow cytometry, and DNA fragmentation assay were used to observe the effects of cytokines on apoptosis. The caspase-3 activity was determined with a fluorocytometer. **RESULTS:** Irradiated Tf-1 cells showed typical morphological characteristic of apoptosis demonstrated by transmission electron microscopy and were accumulated in G_0/G_1 phase. In the groups treated with growth factors after irradiation, three cytokines significantly increased the viability rate, distinctly decreased the apoptosis rate and the proportion of DNA fragmentation. When Tf-1 cells were irradiated by γ irradiation, caspase-3 activity was increased at different time points. In comparison with the control group in which no growth factor was added after the cells were irradiated, the caspase-3 activity of irradiated Tf-1 cells was significantly inhibited by addition of the above cytokines. Thirty-six hours after irradiation, in the control group, GM-CSF, IL-3, GM-CSF and IL-3 in combination, and two GM-CSF/IL-3 fusion protein groups, the apoptosis rate was 73 %, 11 %, 15 %, 13 %, 12 %, and 13 %. The percent of fragmented DNA was 36 %, 19 %, 18 %, 14 %, 13 %, and 14 %. The fluorescence intensity was 16923, 5529, 6581, 5322, 5426, and 5485. **CONCLUSION:** GM-CSF, IL-3, and GM-CSF/IL-3 fusion protein could protect Tf-1 cells from apoptosis induced by γ irradiation. After Tf-1 cells were irradiated, the caspase-3 activity was significantly increased but was dramatically decreased by the above cytokines. The remarkable inhibition of caspase-3 activity may be one of the mechanisms of these hematopoietic growth factors exerting their anti-apoptotic effects.

INTRODUCTION

Recently, there have been ample literatures on the construction and application of recombinant hematopoietic growth factors. Among these growth factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) has a significant promotion on the neutrophil regeneration in sublethally irradiated rhesus monkeys[1]. Interleukin-3 (IL-3) may enhance the hematopoietic function 10 times more in irradiated mice than

[1] Correspondence to Prof Ming-hui YAO.
Phn 86-21-5423-7715. Fax 86-21-5423-7715.
E-mail mhyao@shmu.edu.cn
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in normal mice, and increase the counts of erythrocytes and platelets[2]. The earliest GM-CSF/IL-3 fusion protein consisting of both recombinant human GM-CSF and IL-3 domains was developed by Curtis *et al* in 1991 and was expressed in yeast[3]. The rationale for constructing such a molecule was based on the different and complementary hematopoietic effects of these two cytokines *in vivo*. This new protein has been shown to have biological properties of both GM-CSF and IL-3 *in vivo* tests[4]. In preliminary sublethally irradiated rhesus monkeys, which subjected to total-body ^{60}Co γ irradiation, GM-CSF/IL-3 fusion protein can distinctly increase the counts of leukocytes and platelets, promote the recovery of suppressive bone marrow (BM), decrease haemorrhage and infection, and thus lengthen the living period[5]. Many phase I, II, III clinical trials have demonstrated that GM-CSF/IL-3 fusion protein administered to cancer patients before or following chemotherapy could also elicit the above effects on neutrophil and platelet counts and increase bone marrow cellularity and the percentage of BM cells in S phase. In the future, it may be used as an adjunct treatment in patients undergoing cytopenias following high-dose chemotherapy, radiotherapy and bone marrow transplantation, and used in a mobilization of peripheral-blood progenitor cells and subsequent hematopoietic engraftment. The GM-CSF/IL-3 fusion protein is well tolerated, with the most common side-effect being erythema at the injection site and the mild constitutional symptoms[4,6,7]. Although many researchers reported the results of its clinical trials, few studies focused on the effects and mechanisms of this fusion protein. The present studies were carried out to explore the effects of cytokines GM-CSF, IL-3 and GM-CSF/IL-3 fusion protein on apoptosis of Tf-1 cell line induced by γ irradiation and to probe into the molecular mechanisms of effects of these cytokines on recovery of suppressed bone marrow by radio-therapy for cancer patients.

MATERIALS AND METHODS

Drugs and reagents Recombinant human (rh) GM-CSF was purchased from Scheuing-Plough Pharmaceutical Co, with GM-CSF/IL-3 fusion protein, a gift from foreign laboratory. These two products are lyophilized powders, and were dissolved in sterile injection water to make stock solution and stored in aliquots at -20 ºC. rhIL-3 (Pharmagen Company) was dissolved in PBS and was also stored in aliquots at -20 ºC. MTT, propidium iodide (PI), RNase A, RPMI medium 1640, Tris, edetic acid, Triton-X 100, trichloroacetic acid (TCA) and diphenylamine were purchased from Huamei company; New-born calf serum (NCS), from Hangzhou Sijiqing Co; Caspase-3 Fluorometric Assay kit, from RD systems, Inc.

Cell culture The Tf-1 cell line was a kind gift from LIU Xin-Yuan academician of the Biochemical Institute, the Chinese Academy of Sciences. The Tf-1 cell line was maintained in RPMI-1640 containing GM-CSF 5 μg/L, 10 % NCS, *L*-glutamine 2 mmol/L, Hepes 5 g, $NaHCO_3$ 2 g, penicillin 100 kU/L, streptomycin and kanamycin 100 mg/L, respectively. The cells were grown at 37 ºC in a humidified 5 % CO_2 incubator. Those in logarithmic period were applied to experiments. Cells were washed twice with media lacking GM-CSF and new born serum. Then the concentration of Tf-1 cells was adjusted at 2.5×10^8/L with media lacking GM-CSF[8,9].

Cell irradiation Tf-1 cells were irradiated at a rate of 0.93 Gy/min in a Gammacell-40 ^{137}Cs irradiator. The total irradiation dose was 30 Gy. After irradiation within 30 min, different growth factors were added into the media. The cells were separated into six groups: control; GM-CSF 10 μg/L; IL-3 10 μg/L; GM-CSF 10 μg/L and IL-3 10 μg/L combination; GM-CSF/IL-3 fusion protein 10 μg/L; GM-CSF/IL-3 fusion protein 100 μg/L.

Viability examination The irradiated Tf-1 cells after being added different growth factors were seeded into 96-well plates, cells in a volume of 100 μL at 2.5×10^4 were added into each well (4 wells for each group). There were blank wells in each plate, into which only media were added. The plates were incubated at 37 ºC in a humidified 5 % CO_2 atmosphere. MTT 20 μL at the concentration of 5 g/L was added into each well at 0 h, 12 h, 24 h, 36 h, 48 h, and 60 h of incubation. The lysis solution of crystals (10 % SDS-5 % isobutanol-0.012 mol/L HCl) 100 μL was added into each well after a 4 h-incubation. After incubation overnight at 37 ºC, the absorbance of each well was measured at the wavelength of 570 nm. The four absorbance values were averaged, with the ratio of viable cells calculated by the proportion of absorbance value at different moments to absorbance value at 0 h moment[10]. All experiments were repeated at least three times.

Electron microscopic observation of morphology After 24 h exposure to no exogenous growth factors following irradiation, Tf-1 cells (1×10^6) were cen-

trifuged for 15 min at 1500×*g*, fixed with 3 % glutaraldehyde, and rinsed twice with PBS. Preparations were then postfixed in 1 % osmium tetroxide and dehydrated in ethanol. After that, the cells were embedded in Epon 812. Samples were then ultramicro-tomed. The thin sections were routinely stained and examined by transmission electron microscopy[11].

Cell-cycle distribution and rate of apoptosis analysis by flow cytometry After irradiation and addition of different growth factors for 39 h and 72 h, the Tf-1 cells (1×10^6) were obtained from centrifugation for 5 min at 1000×*g*. The cells were washed twice with PBS and centrifuged, and the pelleted cells were fixed and permeabilized with 70 % ice-cold ethanol in PBS at 4 ºC for 1 h - 72 h. The fixed cells were pelleted, resuspended in 1 mL PBS, and incubated at 37 ºC for 30 min with 100 mg/L RNase A. Then, propidium iodide (PI) 20 mg/L was added into the cell suspension, and then placed it in dark at 4 ºC for another 30 min. After the cell suspension was filtered with nylon film to eliminate any cell clumps, the cell cycle status and DNA content were determined with a FACS Calibur (Becton Dickinson)[11].

Diphenylamine quantification of DNA fragmentation Irradiated Tf-1 cells (2×10^6) were washed twice with PBS and resuspended in hypotonic lysis buffer 400 μL. After slowly rolling for 30 min, crude lysate was centrifuged at 13 000×*g* for 10 min. Fragmented DNA is soluble and remains in the supernatant and intact insoluble genomic sized DNA remains in the pellet. TCA was added into two fractions (final concentration of TCA was 12.5 %) and the mixture was stored at 4 ºC overnight. These sections were then centrifuged at 13 000×*g* for another 10 min, resuspended in 5 % TCA 80 μL and hydrolyzed at 90 ºC for 30 min. After centrifugation at 2000×*g* for 10 min, the supernatant was extracted. $HClO_4$ 20 μL and diphenylamine reaction solution 250 μL were added into the above samples and the mixture were incubated at 50 ºC for 2 h. Absorbance was examined at the wave length of 570 nm. The percent of fragmented DNA was evaluated by determining the ratio of supernatant DNA to total DNA (supernatant plus pellet DNA)[12,13].

Analysis of caspase-3 activity Tf-1 cells were grown either in the presence of growth factors (GM-CSF, IL-3, and GM-CSF/IL-3 fusion protein) or in the absence of any exogenous growth factors for 12 h and 36 h after γ irradiation and then the enzymatic activity of caspase-3 was determined using a caspase-3 Fluorometric Assay kit as suggested by the manufacturer. Briefly, 2×10^6 cells were washed once with cold PBS and 50 μL cold lysis buffer was added into the pelleted cells. The cell lysate was incubated on ice for 10 min, centrifuged at 4 ºC and then the whole cell proteins were extracted. The content of protein was decided by the Lowry protein quantitative method. Each enzymatic reaction for caspase-3 activity in a 96 well microplate requires 100 μg protein, 50 μL reaction buffer and 5 μL caspase-3 special fluorogenic substrate (DEVD-AFC). The reactions were incubated at 37 ºC for 1 h-2 h and the fluorescence signal was detected with a fluorescent microplate reader (POLAR star, Galary bMG) using filters at excitation wave length at 390 nm and emitted wave length at 510 nm-520 nm. The level of caspase-3 enzymatic activity in a cell lysate is directly proportional to the fluorescence signal density[8,14,15].

RESULTS

Effect of growth factors on Tf-1 cell proliferation Under light microscopy, after exposure to GM-CSF, IL-3, and GM-CSF/IL-3 fusion protein for 48 h, the proliferation of Tf-1 cells was enhanced in different degrees (Fig 1).

Effect of growth factors on viability rate of Tf-1 cells After γ irradiation by ^{137}Cs, the viability rates of Tf-1 cells were increased at 36 h by GM-CSF/IL-3 fusion protein (100 μg/L), IL-3, and GM-CSF plus IL-3. When cells were grown for 72 h, 84 h, and 96 h, the viability rates were increased in all cytokine-treated groups (Tab 1).

Morphology Transmission electron microscopy studies indicated that the Tf-1 cells demonstrated characteristic morphology of apoptosis when incubated in the absence of GM-CSF for 24 h after γ irradiation: the loss of microvillus projections, the irregular change of the cell shape, wrinkled but structurally intact cytoplasmic organelles, marginated and condensed nuclear chromatin forming the shape of crescents or lump. These changes did not occur in cells incubated with GM-CSF. That the Tf-1 cells have rich nuclear chromatin and cytoplasma suggests they are of immature features (Fig 2).

Apoptosis and cell cycle examined by flow cytometry When we washed Tf-1 cells with media lacking GM-CSF, the cells were not damaged. The apoptosis rate of irradiated cells was (3.3±0.7) % at 1 h

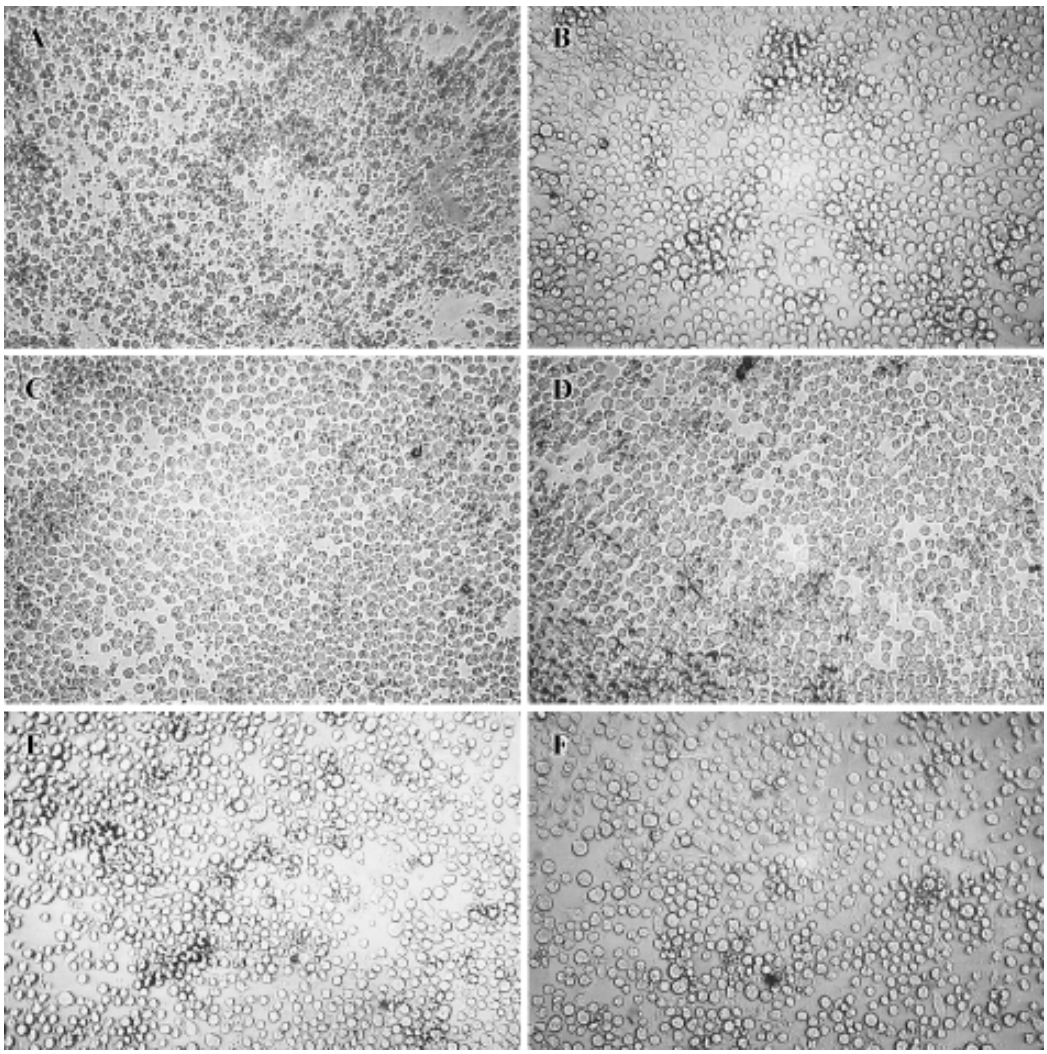

Fig 1. Light microscopic observation of Tf-1 cells after exposure to cytokines for 48 h (10×10). (A) In the medium alone; (B) Addition of GM-CSF 10 μg/L; (C) Addition of GM-CSF/IL-3 fusion protein 10 μg/L; (D) Exposure to GM-CSF/IL-3 fusion protein 100 μg/L; (E) Addition of IL-3 10 μg/L; (F) Exposure to GM-CSF 10 μg/L plus IL-3 10 μg/L.

Tab 1. Effects of cytokines on the viability rate (%) of Tf-1 cells irradiated by ^{137}Cs. *n*=4-6. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* the control group at the same time point.

	Control (with medium alone)	GM-CSF (10 μg/L)	Fusion protein (10 μg/L)	Fusion protein (100 μg/L)	IL-3 (10 μg/L)	GM-CSF (10 μg/L) +IL-3 (10 μg/L)
12 h	87±25	94±31[a]	91±32[a]	95±32[a]	97±38[a]	91±35[a]
24 h	94±26	131±36[a]	124±33[a]	130±42[a]	124±30[a]	127±30[a]
36 h	132±25	164±32[a]	173±40[a]	179±38[b]	180±45[b]	179±52[b]
48 h	168±34	198±47[a]	192±30[a]	194±37[a]	189±34[a]	206±35[a]
60 h	156±30	203±40[a]	196±30[a]	204±38[b]	200±43[a]	202±35[a]
72 h	116±19	188±52[c]	179±49[b]	178±60[b]	172±42[b]	175±18[b]
84 h	80±38	196±62[c]	176±36[b]	195±49[c]	203±32[c]	178±65[b]
96 h	65±18	216±43[c]	198±25[c]	210±40[c]	203±23[c]	200±18[c]

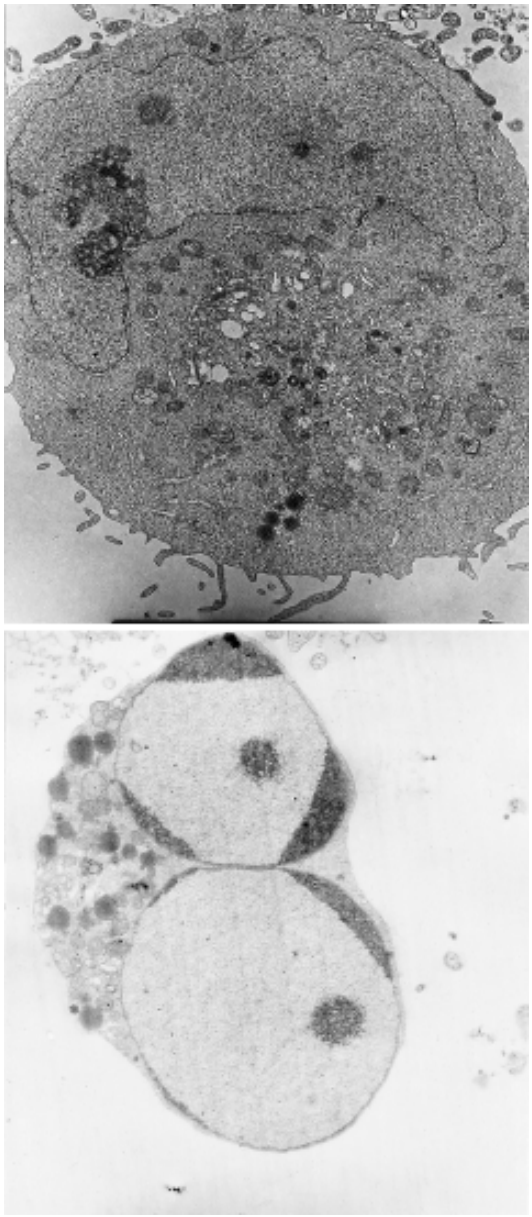

Fig 2. Transmission electron microscopy of Tf-1 cells cultured for 24 h in the presence of GM-CSF (top) and absence of GM-CSF (bottom) after irradiation by ^{137}Cs.

after irradiation and that of non-irradiated normal cultured cells was (4.0±0.8) %. The results showed no difference between the two groups. The cell number with subdiploid DNA was increased with time by either GM-CSF-withdrawal or irradiation. But there were significant differences between these two groups at different time points (Tab 2). Tf-1 cells were cultured in the presence or absence of cytokines GM-CSF, IL-3 and GM-CSF/IL-3 fusion protein for 39 h and 72 h after γ irradiation. A significantly decreased proportion of cells with subdiploid DNA was observed in the groups containing different growth factors compared to those

Tab 2. The apoptosis rate (%) of irradiated Tf-1 cells. *n*=3-4. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

	24 h	39 h	48 h
Control group	21.2±0.5	56±9	72±11
Irradiation group	38±3^{b}	74±3^{b}	89.8±1.2^{c}

Control group=Normal cells deprived of GM-CSF.

cells without exposure to these agents ($P<0.01$). We did not find difference between the cytokine groups at 39 h or 72 h after irradiation. Results did not show concentration-dependent reduction in the two GM-CSF/IL-3 fusion protein groups (Fig 3). Thirty-nine hours after irradiation, the percent of Tf-1 cells in G_0/G_1 was increased and that in S and G_2/M was decreased compared to the normal non-irradiated ones. Tf-1 cells showed remarkable G_0/G_1 arrest after γ irradiation. Compared with the control, the percent of Tf-1 cells in G_2/M phase was increased in all cytokine-treated groups. This result implied that the different cytokines could counteract the cells from apoptosis via deducting G_0/G_1 arrest and leading more arrested cells into G_2/M phase[16]. At 72 h after irradiation, the percent of cells in different cycle phases could not be detected by the same instrument. No further statistical analysis was conducted (Tab 3).

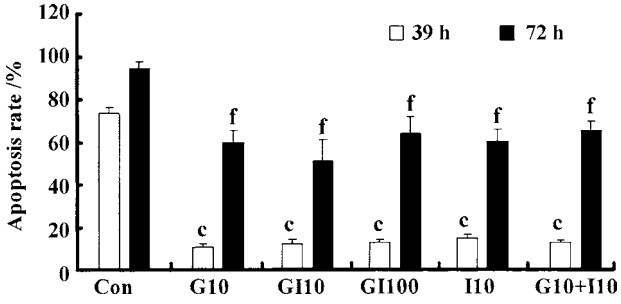

Fig 3. Comparison of anti-apoptotic effects of cytokines on Tf-1 cells after ^{137}Cs irradiation by flow cytometry. *n*=3. Mean±SD. $^{c}P<0.01$, $^{f}P<0.01$ *vs* the control group at 39 h and 72 h, respectively. (Con) Control; (G10) GM-CSF 10 μg/L; (GI10) GM-CSF/IL-3 fusion protein 10 μg/L; (GI100) GM-CSF/IL-3 fusion protein 100 μg/L; (I10) IL-3 10 μg/L; (G10+I10) GM-CSF 10 μg/L+IL-3 10 μg/L.

DNA fragmentation To quantify the effects of different cytokines on the degree of DNA fragmentation, DNA was separately isolated from the supernatants and

Tab 3. Effect of cytokines on cell cycle distribution of Tf-1 cells after ^{137}Cs irradiation. *n*=3-4. Mean±SD. $^{c}P<0.01$ *vs* Non-irradiated Tf-1 cells. $^{d}P>0.05$, $^{c}P>0.05$ *vs* the control group.

	39 h			72 h		
	G_0/G_1 (%)	G_2/M (%)	S (%)	G_0/G_1 (%)	G_2/M (%)	S (%)
Non-irradiated Tf-1 cells	44±4	22±4	34.6±2.9			
Control (with medium alone)	90±3^{c}	3.3±2.0^{c}	6±3^{c}	ND	ND	ND
GM-CSF (10 μg/L)	85.9±2.7^{d}	8.9±2.5^{e}	5.2±0.4^{d}	74±5	11.9±1.4	14±4
GM-CSF/IL-3 fusion protein (10 μg/L)	85±5^{d}	8.1±1.6^{e}	6±3^{d}	78.8±1.4	14±4	7±5
GM-CSF/IL-3 fusion protein (100 μg/L)	87.0±1.5^{d}	8.4±1.4^{e}	4.6±0.2^{d}	77±4	11.7±2.5	11.5±1.3
IL-3 (10 μg/L)	86.1±1.0^{d}	8.4±1.3^{e}	5.5±0.5^{d}	74±3	11.5±0.7	15±4
GM-CSF (10 μg/L)+IL-3 (10 μg/L)	85.0±0.2^{e}	8.9±1.1^{e}	6.1±0.8^{d}	74±3	11.4±0.1	14.2±2.9

ND means could not be measured.

pellets of cell lysates. After 36 h of irradiation, the proportion of DNA fragmentation was significantly inhibited by GM-CSF, IL-3, GM-CSF plus IL-3, and GM-CSF/IL-3 fusion protein compared with the control group. There has statistical difference between the GM-CSF plus IL-3 group and the GM-CSF or IL-3 alone group ($P<0.05$). The differences between the two concentration fusion protein groups and the GM-CSF or IL-3 alone group were also remarkable ($P<0.05$). These results indicated that, compared with GM-CSF or IL-3 alone, the fusion protein or combination of the two cytokines have slightly strong effect in decreasing the DNA fragmentation (Fig 4).

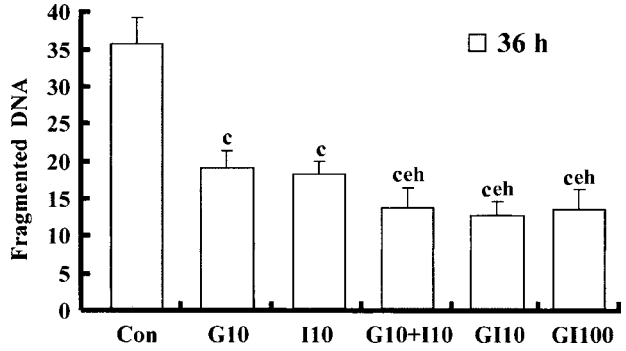

Fig 4. Inhibitory effect of cytokines on the percent of fragmented DNA of irradiated Tf-1 cells. *n*=4. Mean±SD. $^{c}P<0.01$ *vs* control. $^{e}P<0.05$ *vs* G10. $^{h}P<0.05$ *vs* I10. (Con) Control; (G10) GM-CSF 10 μg/L; (I10) IL-3 10 μg/L; (G10+I10) GM-CSF 10 μg/L+IL-3 10 μg/L; (GI10) GM-CSF/IL-3 fusion protein 10 μg/L; (GI100) GM-CSF/IL-3 fusion protein 100 μg/L.

Activity of caspase-3 After irradiation, the caspase-3 activity in Tf-1 cells was enhanced remarkably. The activities of the irradiated cells were 4.5 times and 7.6 times at 12 h and 36 h higher than those of the non-irradiated control group respectively. The increased caspase-3 activity in Tf-1 cells was remarkably inhibited by GM-CSF, IL-3, GM-CSF/IL-3 fusion protein, and GM-CSF plus IL-3 at 12 h and 36 h after irradiation. In the above cytokine groups, the caspase-3 activities were 31.5 %-38.9 % of that in the control group (Fig 5).

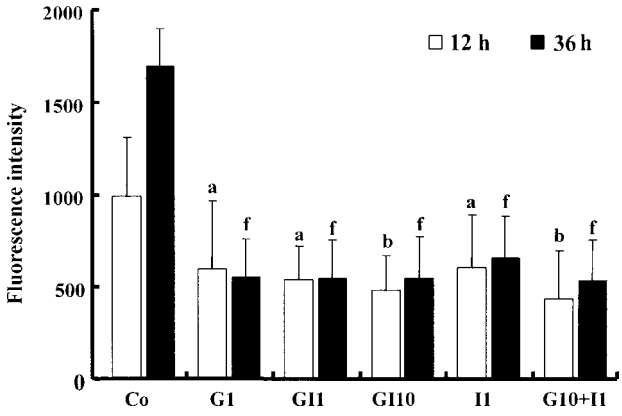

Fig 5. Inhibitory effect of cytokines on caspase-3 activity of irradiated Tf-1 cells. *n*=3. Mean±SD. $^{a}P>0.05$, $^{b}P<0.05$ *vs* control at 12 h. $^{f}P<0.01$ *vs* control at 36 h. (Con) Control; (G10) GM-CSF 10 μg/L; (GI10) GM-CSF/IL-3 fusion protein 10 μg/L; (GI100) GM-CSF/IL-3 fusion protein 100 μg/L; (I10) IL-3 10 μg/L; (G10+I10) GM-CSF 10 μg/L+IL-3 10 μg/L.

DISCUSSION

Apoptosis is a nonspecific physiological process which has been showed for hematopoietic cells following cytokines deprivation or exposure to chemical agents or radiation[17]. Human Tf-1 myeloid leukaemia cell line

was derived from a patient with erythroleukemia, and the cell's proliferation is dependent on three hemopoietic growth factors: GM-CSF, IL-3 and erythropoietin (EPO). Ultrastructural studies have revealed some immature features in this cell line. Our studies also demonstrated this phenomenon. Tf-1 cells are multipotential as they can differentiate into more mature erythroid, monocytic or megakaryocytic cells. As a result, this cell line represents a model for analysis for the effects of some cytokines and oncogene expression on human hematopoietic cell growth and differentiation[18,19].

GM-CSF/IL-3 fusion protein is a genetically engineered new type of stimulating hematopoietic agent. This *in vitro* study was aimed at investigating the effects and mechanisms of several hemapoietic growth factors, especially GM-CSF/IL-3 fusion protein on apoptosis of Tf-1 cells trigged by radiation in an attempt to counteract this apoptosis process for therapeutical value. GM-CSF, IL-3, and GM-CSF/IL-3 fusion protein can promote the survival and stimulate the proliferation of Tf-1 cells to some extent. Tf-1 cells presented typical morphologic changes of apoptosis demonstrated by electron transmission microscopy after they were irradiated by $^{137}C_S$. The data presented here demonstrated that hematopoietic growth factors such as GM-CSF, IL-3 and GM-CSF/IL-3 fusion protein could protect Tf-1 cells to some extent from apoptosis induced by γ irradiation. The viability rate of Tf-1 cells can be markedly increased, and the apoptosis rate significantly be reduced by these growth factors compared to that of the controls. Tf-1 cells showed remarkable G_0/G_1 arrest after γ irradiation. Thus γ irradiation could cause a decreased DNA synthesis and mitosis and induce apoptosis.

Caspases are a family of cysteine-kinase proteases implicated in the biochemical and morphological changes that occur during apoptosis. It has been proved that there are at least 13 kinds of caspases in the human body. Caspase-3 is one of the most important caspases in signal pathways in apoptosis[20]. The activated caspase can cleave DNA by adjusting the CAD (caspase-activated deoxyribonuclease) and ICAD (inhibitor of CAD)[21].

In the present research, we found that when Tf-1 cells were irradiated by γ ray for 39 h and 72 h, the apoptosis rate increased gradually, so did the caspase-3 activity increase after 12 h and 36 h of irradiation. The above results showed that caspase-3 activation was involved in the processes of irradiation-inducing apoptosis of Tf-1 cells. Hematopoietic growth factors (GM-CSF, IL-3, and GM-CSF/IL-3 fusion protein) added into irradiated Tf-1 cells produced a remarkable decline in apoptosis rate and percent of fragmented DNA as well as in caspase-3 activity. These results suggested that the markedly inhibited caspase-3 activity may be one of the mechanisms of these hematopoietic growth factors exerting their anti-apoptotic effects.

We found that Tf-1 cells could be rescued from γ irradiation-induced apoptosis by addition of GM-CSF, which are consistent with the observation reported by Kelly[9], and we found that IL-3, GM-CSF and IL-3 in combination, and GM-CSF/IL-3 fusion protein protected Tf-1 cells from the apoptosis induced by γ irradiation. We also found that γ-irradiation can lead to increase in the proportion of DNA fragmentation and caspase-3 activity, which can be effectively inhibited by such different cytokines as GM-CSF, IL-3, GM-CSF and IL-3 in combination, and GM-CSF/IL-3 fusion protein.

In this *in vitro* study, we did not find the fusion protein had more potent anti-apoptotic effect on the irradiated Tf-1 cells than GM-CSF, IL-3, or the combination of these two cytokines as observed *in vivo* experiments[1,6,7]. One possible reason is that, larger molecular fusion protein have difficulty in transferring into Tf-1 cells *in vitro* environment, whereas in whole body, there may have some enzymes helping to break down the larger molecule into more active particles.

The protective effects of these growth-promoting factors on apoptosis of irradiated Tf-1 cells in the present study implies that bone marrow progenitor cells might be protected by appropriate hematopoietic agents administered *in vivo* after radiotherapy on cancers.

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