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Phorbol Ester Facilitates Apoptosis in Murine Fibroblasts Pretreated by Mild Ultraviolet Radiation

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Although phorbol 12-myristate 13-acetate (PMA) inhibits apoptosis and promotes the growth of some types of cells, it induces apoptosis in other cells. We evaluated the apoptotic effects of PMA on murine fibroblasts (L-929) that had been exposed to ultraviolet-B (UV-B) radiation at 312 nm, which promotes tumor cell growth. Exposure to PMA alone did not induce Fas, Fas-L, or apoptosis. Cells exposed to mild UV-B irradiation (80 J/m²) alone exhibited a slight expression of Fas and Fas-L 36 to 48 h after the exposure, and exhibited apoptosis as evidenced by DNA fragmentation 72 h after exposure. The addition of PMA (0.8×10^{-5} to 3.2×10^{-5} M) to the medium 24 h after the UV-B exposure markedly and dose-dependently enhanced these cell responses. Confluent untreated cells, cells cocultured with PMA, and cells cocultured with PMA for 24 h after the UV-B exposure consistently expressed mRNAs for wild-type *p53*, *bcl-2*, and *ICE*. Expression of c-*myc* mRNA was initially observed, but became undetectable in the cells cocultured for 24 h with a high concentration of PMA (3.2×10^{-5} M) following UV-B exposure. Such cells subsequently exhibited the maximal apoptotic response. We conclude that mild exposure to UV-B altered murine fibroblast cells in such a way as to facilitate their death by apoptosis upon addition of PMA.

Key words: apoptosis, Fas, Fas-ligand, phorbol myristate acetate, ultraviolet.

Our environment contains numerous physical and chemical carcinogens whose actions and interactions are of considerable interest (*1*). The tumor-promoting agent phorbol 12-myristate 13-acetate (PMA) activates such protooncogenes as c-*myc*, *ras*, c-*fos*, and c-*jun* (*2-4*) and may induce the transformation, proliferation, and differentiation of the cell (*2*). PMA inhibits apoptosis in many types of cells. It has been reported that PMA protects bovine aortic endothelial cells against the apoptosis caused by exposure to gamma rays (*5*), by withdrawal of the growth factor from normal myeloid precursor cells and from myeloid leukemic cells clone 12 (*6*), and by exposure to TNF-α (*7*). PMA has also been shown to induce apoptosis in several types of cancer cells (*8-14*), and thus exhibits diverse effects on apoptosis. Its effect in this respect may depend on the type of cell involved (*12*).

Ultraviolet (UV) radiation, a potent tumor promoter, activates such protooncogenes as c-*fos*, c-*jun*, and *p53* (*2, 15*). Excessive exposure to UV radiation causes skin cancer (*16*). The risk of harmful exposure to UV radiation is gradually increasing with the continuing erosion of the ozone layer in the stratosphere (*17*). Thus an increased incidence of skin cancer can be expected. However, a high dose of UV induces apoptosis (*18*).

It is not known how cells exposed to a mild dose of UV and then to a tumor promoter, such as PMA, may behave in terms of apoptosis. Accordingly, we studied the effects of PMA on murine fibroblasts (L929) pretreated with a mild dose of UV-B (312 nm). This dose of irradiation appears to be relevant to human exposure (*19*).

MATERIALS AND METHODS

Cell Line and Cell Culture—Murine fibroblast L-929 cells (NCTC clone 929) were purchased from Dainippon Pharmaceutical (Osaka) and maintained in Eagle's minimum essential medium (MEM) (Nissui Pharmaceutical, Tokyo) containing 10% fetal calf serum (FCS).

Conditions of UV-B Exposure and of PMA Addition—Confluent L-929 cells ($3.1\text{-}3.4\times10^5$/ cm²) in 25 cm² tissue culture flasks (Corning, NY, USA) were irradiated with UV-B (312 nm, 80 J/m²) using a UV-B generator (Atto, DT-20MP, Tokyo) 24 h prior to coincubation with PMA (Sigma, St. Louis, USA). PMA was added to the culture medium at a concentration ranging from 0.0 to 3.2×10^{-5} M. PMA was used as a 3.2 mM solution in dimethylsulfoxide (DMSO) and MEM (DMSO: MEM, 1:5).

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Abbreviations: DMSO, dimethylsulfoxide; Fas-L, Fas-ligand; FCS, fetal calf serum; MEM, Eagle's minimum essential medium; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate; RT-PCR, reverse-transcription coupled polymerase chain reaction; UV-B, ultraviolet-B.

Analysis of DNA Fragmentation and Cell Count—Analysis of DNA fragmentation was performed separately in the cells attached to the wall of the flask and in the detached cells that were suspended in the culture medium. The detached cells were collected by centrifugation ($1,500 \times g$, 15 min, 4°C) and washed with phosphate-buffered saline (PBS) without magnesium and calcium. Attached cells were washed with PBS, trypsinized in PBS with 0.02% of EDTA, neutralized with FCS, and collected by centrifugation ($700 \times g$, 10 min, 4°C). DNA from these cells was purified according to the method previously reported (*20*). Purified DNA was electrophoresed on 1.5% agarose gel.

The collected cells were stained with Turk's solution and counted with a Burker-Turk's cell counter.

Analysis of Fas and Fas-Ligand (Fas-L) Expression by Fluorescence Flow Cytometry—The untreated and the variously treated cells in the culture flasks were washed twice with PBS. The cells attached to the flask wall were detached with a cell scraper, dispersed by gentle pipetting, and passed through a 45-μm filter (Millipore, MA, USA). A 20 μg/ml of anti-mouse monoclonal rat Fas IgG antibody (BML Laboratory, Nagoya) or 20 μg/ml of anti-mouse rabbit polyclonal Fas-L IgG antibody (mouse and rat Fas-L reactive, Santa Cruz Biotechnology, MA, USA) was added to $3\text{-}5 \times 10^5$/ml of cell suspension in PBS and incubated for 1 h at 4°C. The cells were washed twice with PBS, then stained with 10 μg/ml of the FITC-conjugated $F(ab')_2$ fragment of anti-rat rabbit IgG (Organon Tecnica, Durham, NC, USA) (*21*). Fluorescence flow cytometry analysis was then performed (Epics XL System II, Coulter, Tokyo).

Cell Cycle Analysis—Analysis of the cell cycle was performed according to the method previously reported (*22*). Propidium iodide fluorescence was measured by FACS Calibur with the use of Modfit-LT software (Becton Dickinson Immunocytometry Systems, NJ, USA).

Analysis of mRNAs for Wild Type p53, bcl-2, c-myc, and ICE—RNA was purified by Ultraspec III (Biotecx Laboratory, Houston, USA). mRNAs for wild type *p53*, *bcl-2*, c-*myc*, and *ICE* were amplified by the reverse-transcription coupled polymerase chain reaction (RT-PCR), using mAPO1-MPCR kits (Maxim Biotech., San Francisco, USA).

The RT-PCR primers for *p53* were: 5′-CGG AGG TCG TGA GAC GCT G-3′ and 5′-CAC ATG TAC TTG TAG TGG ATG GTG G-3′. The RT-PCR primers for *bcl-2* were: 5′-CAG CTG CAC CTG ACG CCC TT-3′ and 5′-CCC AGC CTC CGT TAT CCT GGA-3′. The RT-PCR primers for c-*myc* were: 5′-CAG CAG AGC GAG CTG CAG CC-3′ and 5′-CTG TCT TTG CGC GCA GCC TG-3′. The RT-PCR primers for *ICE* were: 5′-GTA CAC GTC TTG CCC TCA TTA TCT G-3′ and 5′-GGT TGT TCA AAT GAA AAT CGA ACC-3′. The theoretical base pairs (bp) of RT-PCR products of *p53*, *bcl-2*, c-*myc*, and *ICE* were 205, 235, 374, and 655 bp, respectively. All primer sequence data were provided by Maxam Biotec. The RT-PCR procedures were performed according to the manufacturer's instructions.

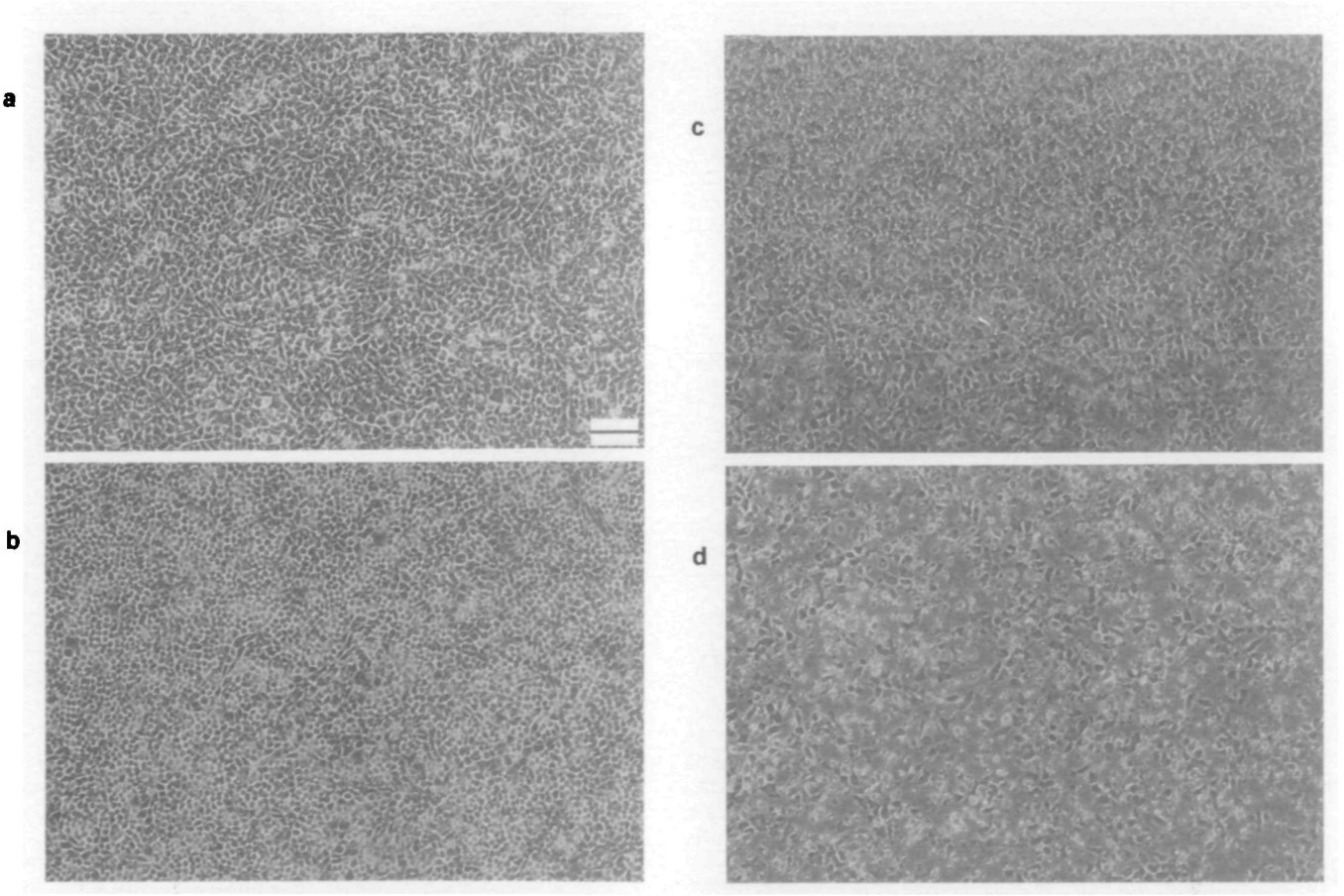

Fig. 1. **Apoptosis induced by PMA in cells pretreated with UV-B.** a, untreated cells (corresponds to line 1 at 72 h in Fig. 2); b, cells coincubated with 3.2×10^{-8} M PMA for 48 h (corresponds to line 2 at 72 h in Fig. 2); c, cells 72 h after UV-B exposure (corresponds to line 3 at 72 h in Fig. 2); d, cells preteated with UV-B and coincubated with 3.2×10^{-8} M PMA for 48 h (corresponds to line 6 at 72 h in Fig. 2).

RESULTS

Light Microscopic Findings and Percentage of Cells Attached to Flask Wall over Time—Using light microscopy, we investigated the serial changes in the cells treated with UV-B alone, PMA alone, and with combined UV-B and PMA (Fig. 1). The shape or density of cells coincubated with 3.2×10^{-5} M PMA for 48 h did not differ from those of untreated cells attached to the flask wall (Fig. 1; b *vs.* a). After 72 h of UV-B exposure, a significant number of cells became detached from the flask wall so that a cell-free space was apparent (Fig. 1c). Many of the cells pretreated with UV-B 24 h prior to addition of PMA, then coincubated with 3.2×10^{-5} M PMA for 48 h developed a round shape and became detached from the flask wall (Fig. 1d), which suggested that PMA had facilitated apoptosis in the irradiated cells.

The percentage of cells attached to the flask wall over time is shown in Fig. 2. Untreated cells or cells coincubated with 3.2×10^{-5} M PMA did not become detached from the wall during observation (Fig. 2, lines 1 and 2). Cells exposed to UV-B began to become detached from the wall

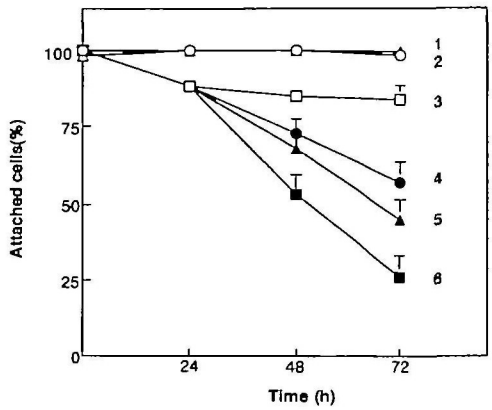

Fig. 2. **Effect of UV-B and PMA on the cell rate attached to the flask wall.** Irradiation with UV-B was performed at 0 h. PMA was added at 24 h after UV-B exposure. Vertical bar indicates the mean ± standard error (SE) of triplicate experiments. Line 1, untreated cells; line 2, 3.2×10^{-5} M PMA alone; line 3, UV-B alone; line 4, UV-B plus 0.8×10^{-5} M PMA; line 5, UV-B plus 1.6×10^{-5} M PMA; line 6, UV-B plus 3.2×10^{-5} M PMA.

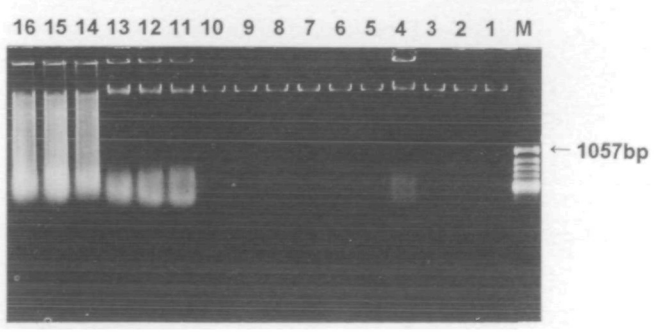

Fig. 3. **Cleavage of DNA in cells treated with UV-B and PMA.** M, marker (ϕX174/*Hin*cII digest); lane 1, untreated cells; lanes 2, 3, and 4, attached cells 24, 48, and 72 h after UV-B exposure, respectively; lanes 5, 6, and 7, attached cells coincubated with 3.2×10^{-5} M PMA for 24, 48, and 72 h, respectively; lanes 8, 9, and 10, attached cells pretreated with UV-B and coincubated with 0.8×10^{-5} M PMA, 1.6×10^{-5} M PMA, and 3.2×10^{-5} M PMA, respectively for 24 h (corresponding to 48 h in Fig. 2); lanes 11, 12, and 13, attached cells pretreated with UV-B and coincubated with 0.8×10^{-5} M PMA, 1.6×10^{-5} M PMA, and 3.2×10^{-5} M PMA, respectively for 48 h (corresponding to 72 h in Fig. 2); lanes 14, 15, and 16, detached and suspended cells in the culture medium in the same experiments as lanes 11, 12, and 13, respectively.

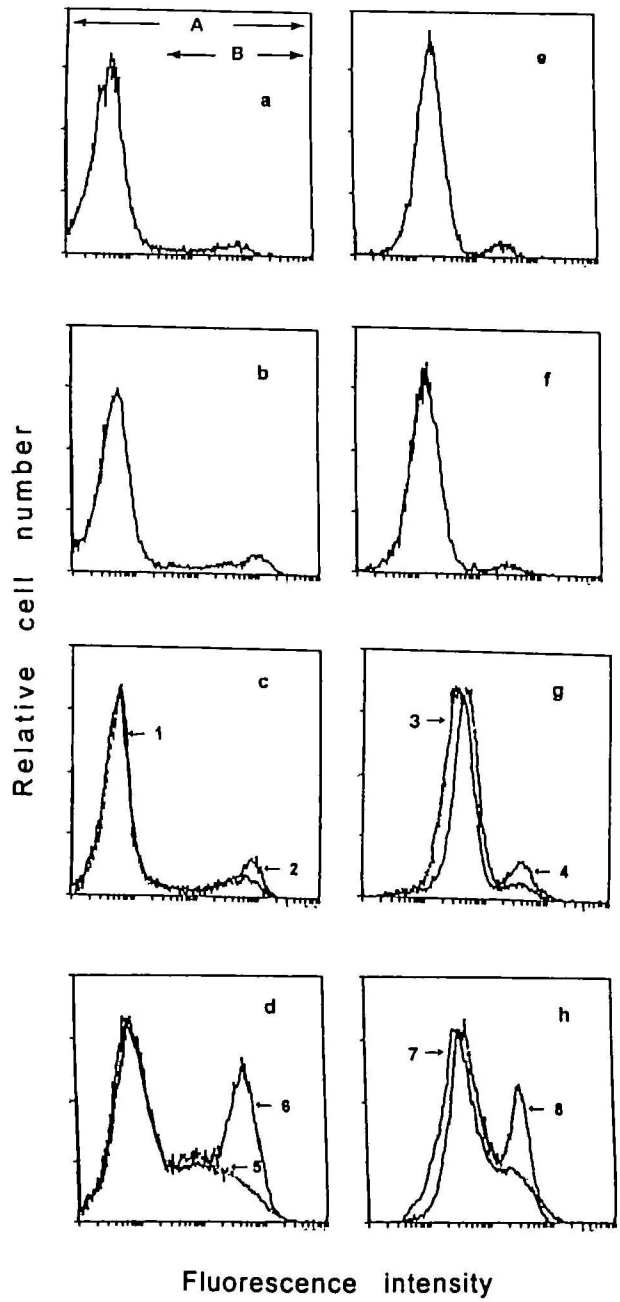

Fig. 4. **Expression of Fas and Fas-L.** a, b, c, and d, experiments for detection of Fas. e, f, g, and h, experiments for detection of Fas-L. a and e, untreated cells; b and f, cells coincubated with 3.2×10^{-5} M PMA for 24 h; c and g, 36 h (curves 1 and 3) and 48 h (curves 2 and 4) after UV-B exposure; d and h, cells pretreated with UV-B 24 h prior to addition of PMA and coincubated with 3.2×10^{-5} M PMA for 12 h (corresponding to 36 h in Fig. 2) (curves 5 and 7) and 24 h (corresponding to 48 h in Fig. 2) (curves 6 and 8).

within 24 h, but the percentage of attached cells did not decrease significantly thereafter (Fig. 2, line 3). When the cells pretreated with UV-B were coincubated with PMA, the percentage of attached cells gradually declined over time and with an increase in the concentration of PMA (Fig. 2, lines 4 to 6).

DNA Fragmentation in Cells Treated with UV-B and/or PMA—We investigated the serial changes in DNA fragmentation in cells treated with UV-B alone, PMA alone, and UV-B and PMA. Since the cells detached from the flask wall and suspended in the medium were considered to have undergone death by apoptosis, we investigated whether they would show DNA fragmentation. Cells that were attached to the flask did not exhibit DNA fragmentation until 48 h after UV-B exposure (Fig. 3, lanes 2 and 3), but exhibited a slight DNA fragmentation 72 h after UV-B exposure (Fig. 3, lane 4). The attached cells coincubated with 3.2×10^{-5} M PMA did not exhibit DNA fragmentation in the absence of pretreatment with UV-B during the 72-h observation (Fig. 3, lanes 5 to 7). The attached cells pretreated with UV-B and coincubated with PMA (0.8 to 3.2×10^{-5} M) for 24 h (corresponding to 48 h after UV-B exposure) did not exhibit DNA fragmentation (Fig. 3, lanes 8 to 10). The attached cells pretreated with UV-B and coincubated with PMA (0.8 to 3.2×10^{-5} M) for 48 h (corresponding to 72 h after UV-B exposure) exhibited stronger DNA fragmentation than did the attached cells treated with UV-B alone (lanes 11 to 13 *vs.* lane 4), suggesting that PMA facilitated apoptosis in the cells pretreated with UV-B. At this time, the cells that had become detached from the flask wall and were suspended in the medium showed a more pronounced fragmentation of DNA (lanes 14 to 16) than did the attached cells (lanes 11 to 13), suggesting that the detached cells were at a more advanced stage of apoptosis.

Time Course of Fas and Fas-L Expression—Cells coincubated with 3.2×10^{-5} M PMA for 24 h or for 36 to 48 h after UV-B exposure exhibited little change in Fas expression (Fig. 4, b and c) compared with untreated cells (Fig. 4a). The addition of 3.2×10^{-5} M PMA markedly increased Fas expression within 12 h in the cells pretreated with UV-B (curve 5 in Fig. 4d *vs.* curve 1 in Fig. 4c) and further increased Fas expression thereafter (curve 6 in Fig. 4d *vs.* curve 2 in Fig. 4c).

The cells exposed to UV-B showed a shift to the right (Fig. 4g), indicating that UV-B exposure increased Fas-L expression within 36 h (curve 3 in Fig. 4g). Fas-L expression was further increased by PMA in the cells pretreated with UV-B (Fig. 4, h *vs.* g).

We investigated the effect of PMA concentration on Fas and Fas-L expression. The relative area, defined as a $B/A\times100$ (areas of A and B are shown in Fig. 4a), was calculated (Fig. 5). PMA increased Fas and Fas-L expression dose-dependently only in cells pretreated with UV-B. Cells treated with UV-B alone exhibited a slight expression of Fas and Fas-L.

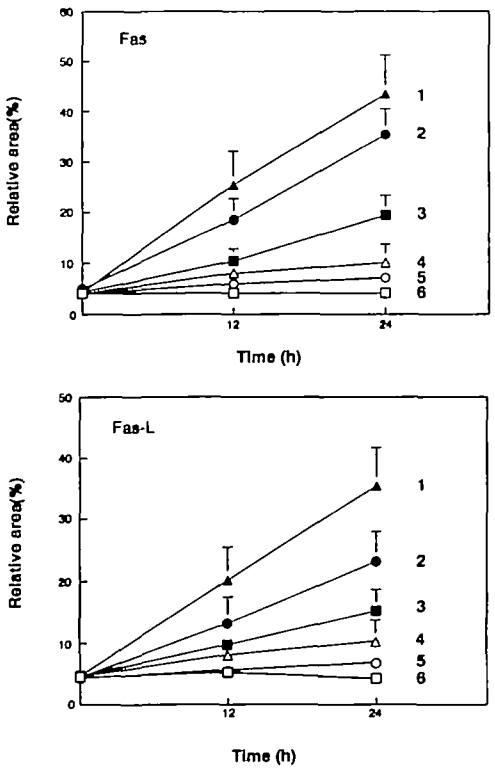

Fig. 5. **Effects of concentration of PMA on expression of Fas (upper panel) and Fas-L (lower panel).** Relative area was defined as a $100\times B/A$. Areas of A and B are depicted in a. Vertical bar indicates the mean±SE of triplicate experiments. Cells were pretreated with UV-B 24 h prior to addition of PMA at 0 h. 1, UV-B plus 3.2×10^{-5} M PMA; 2, UV-B plus 1.6×10^{-5} M PMA; 3, UV-B plus 0.8×10^{-5} M PMA; 4, UV-B alone; 5, 3.2×10^{-5} M PMA alone; 6, untreated cells.

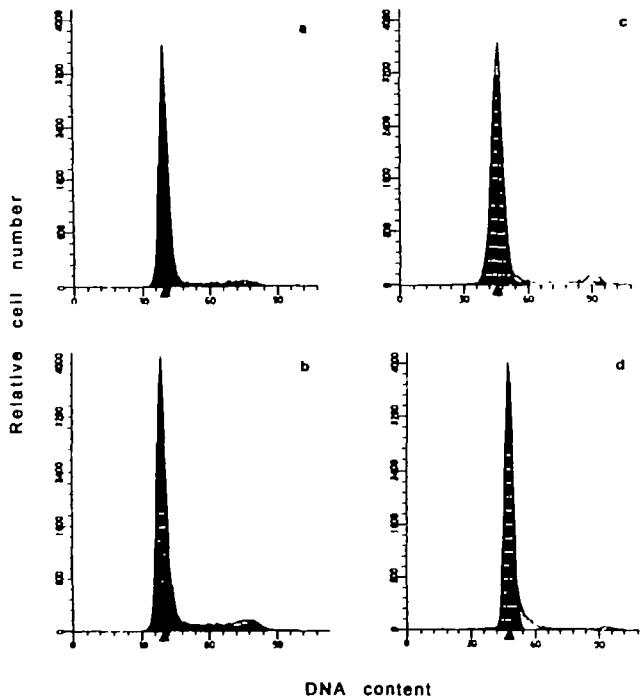

Fig. 6. **Cell cycle analysis by fluorescence flow cytometry.** a, untreated cells (G_0/G_1:91.4%, S:4.9%, G_2/M:3.7%); b, cells coincubated with 3.2×10^{-5} M PMA for 24 h (corresponding to 48 h in Fig. 2) (G_0/G_1: 89.3%, S: 4.1%, G_2/M: 6.6%) in the absence of UV-B pretreatment; c, cells 48 h after UV-B exposure (G_0/G_1: 91.5%, S: 2.9%, G_2/M: 5.6%) in the absence of PMA; d, cells pretreated with UV-B and coincubated with 3.2×10^{-5} M PMA for 24 h (corresponding to 48 h in Fig. 2) (G_0/G_1: 93.7%, S: 2.7%, G_2/M: 3.6%).

Cell Cycle Analysis of the Attached Cells—To examine the relationship between cell cycle and the timing of Fas and Fas-L expressions in cells that have been treated with PMA and/or UV-B and are destined to undergo apoptosis, we analyzed the cell cycle in untreated cells, cells treated with PMA alone, and cells treated with both UV-B and PMA (Fig. 6). Analysis of DNA content revealed that more than 89% of untreated cells, cells coincubated with PMA for 24 h in the absence of UV-B pretreatment, cells 48 h after UV-B treatment in the absence of PMA, or cells treated with UV-B and coincubated with PMA for 24 h were in stage G_0-G_1. Thus, no significant change in the cell cycle had occurred after UV-B and/or PMA treatment at this time. Meanwhile, expressions of Fas and Fas-L occurred (see Fig. 4, d and h), but DNA fragmentation had not occurred yet (see Fig. 3, lanes 8 to 10).

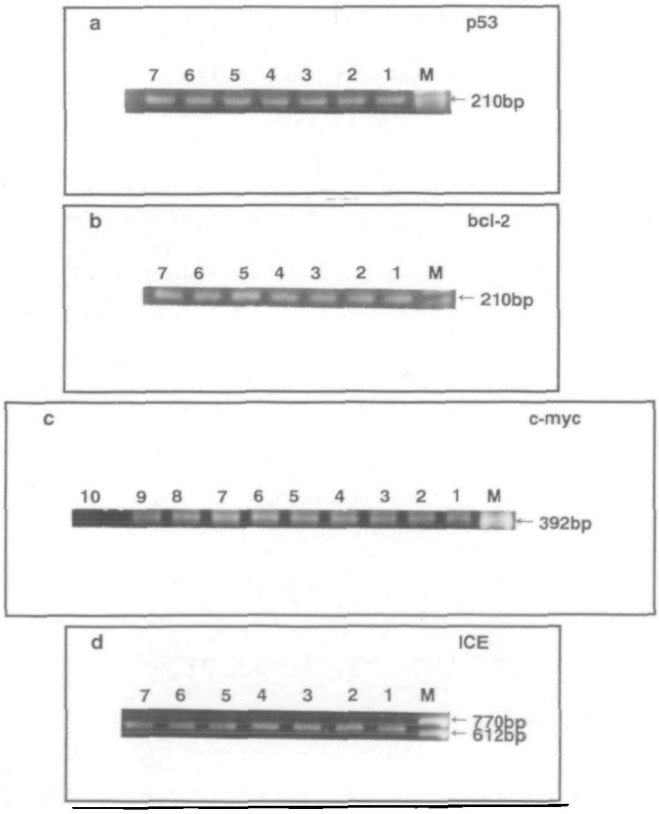

Fig. 7. **Time course of expression of mRNAs for *p53*, *bcl-2*, c-*myc*, and *ICE*.** Explanations of a (*p53*), b (*bcl-2*), and d (*ICE*) are as follows: M, marker (ϕX 174/*Hin*cII digest); lane 1, untreated cells; lane 2, cells coincubated with 3.2×10^{-5} M PMA for 48 h (corresponding to 72 h in Fig. 2); lanes 3 and 4, 24 and 48 h after UV-B exposure, respectively; lanes 5, 6, and 7, cells pretreated with UV-B and coincubated with 0.8×10^{-5} M PMA (lane 5), 1.6×10^{-5} M PMA (lane 6), and 3.2×10^{-5} M PMA (lane 7) for 24 h (48 h in Fig. 2). Explanation of c (c-*myc*) is as follows: M, marker (ϕX174/*Hin*cII digest); lane 1, untreated cells; lane 2, cells coincubated with 3.2×10^{-5} M PMA for 48 h (72 h in Fig. 2); lanes 3 and 4, 24 and 48 h after UV-B exposure, respectively; lanes 5 and 6, cells pretreated with UV-B and coincubated with 0.8×10^{-5} M PMA for 12 h (36 h in Fig. 2) (lane 5), and for 24 h (48 h in Fig. 2) (lane 6); lanes 7 and 8, cells pretreated with UV-B and coincubated with 1.6×10^{-5} M PMA for 12 h (36 h in Fig. 2) (lane 7), and for 24 h (48 h in Fig. 2) (lane 8); lanes 9 and 10, cells pretreated with UV-B and coincubated with 3.2×10^{-5} M PMA for 12 h (36 h in Fig. 2) (lane 9), and for 24 h (48 h in Fig. 2) (lane 10).

Change in Expression of mRNAs for p53, bcl-2, c-myc, and ICE—We investigated the serial changes in the expression of mRNA encoding *p53*, *bcl-2*, c-*myc*, and *ICE* (Fig. 7). All the cells examined under the various conditions expressed mRNAs for *p53*, *bcl-2*, and *ICE* (Fig. 7, a, b, and d). The mRNA for c-*myc* (Fig. 7c) was also detected under all conditions, except for lane 10, which indicated that the expression of c-*myc* mRNA was reduced to an undetectable level by coincubation with 3.2×10^{-5} M PMA for 24 h in the cells pretreated with UV-B.

DISCUSSION

The present study demonstrated that PMA facilitated cell death by apoptosis in murine fibroblasts that had been pretreated with mild UV-B. Animal cells will undergo apoptosis or necrosis if the damage to them is great enough, whereas cells with only mild injury may undergo repair and survive (*23*). Although a high dose of UV induces apoptosis (*18*), mild exposure to UV radiation acts as a physical carcinogen (*2, 15, 16*). The present study demonstrated that the chemical carcinogen PMA, which itself did not induce apoptosis, hampered the repair or rescue of cells injured by a physical carcinogen, UV-B.

It has been reported that PMA protects the cell against the apoptosis induced variously by exposure to TNF-α (*7*), administration of chemotherapy (*27*), or withdrawal of growth factor (*6*), which contradicts the present results. However, pretreatment with a physical carcinogen such as UV radiation was not performed prior to exposure to PMA in these studies (*6, 7, 27*). We attempted to examine the effect of the chemical carcinogen on the cell that have suffered mild injury by UV-B radiation. A few studies have focused on the synergistic or additive effects of physical and chemical carcinogens on the cell behavior. In one report, a brief exposure to PMA (30 ng/ml for 30 min) was found to inhibit the apoptotic degradation of DNA caused by gamma irradiation in bovine aortic endothelial cells (*5*). However, we added PMA to the culture medium 24 h after UV-B exposure and the cells were coincubated with PMA for longer periods. Whether the different results of the earlier study (*5*) and in the present study are attributable to differing experimental conditions or differing cell lines evaluated is not clear.

PMA has been reported to induce apoptosis in several types of malignant cells, such as prostate cancer cells (*8-11*) and leukemic cells (*13-15*). To our knowledge, there are no previous reports of apoptosis induced by PMA in fibroblasts (L-929), although the cells used in the present study were mildly irradiated by UV-B. Because L-929 cells have been shown to be tumorigenic when inoculated in mice previously exposed to X-rays (*28*), these cells may possess some characteristics of cancer cells. However, these cells did not undergo apoptosis in response to PMA in the absence of UV-B pretreatment in the present study. It is possible that the L-929 cells acquire malignant characteristics following UV-B radiation and thus become more susceptible to apoptosis in response to the addition of PMA. In the present study, we used a higher concentration of PMA than in previous studies (*3, 5, 9, 12*). In our preliminary study, we could not detect an apoptotic response in the cells pretreated with UV-B at the usual concentration of PMA (0.1 to 0.2 μM). In addition, the maximum dose, 32

μM PMA, used in this study had a little effect on L-929 cells with respect to apoptotic response in the absence of UV-B pretreatment. We do not yet know whether the L-929 cell is a more resistant strain to PMA than other cells used in previous studies (*3, 5, 9, 12*).

The expression of Fas and Fas-L is important in apoptosis (*24-26*). In the cells treated with UV-B alone, a slight expression of Fas and Fas-L was observed 36 to 48 h after UV-B exposure, with DNA fragmentation occurring in a small number of cells after 72 h. Although PMA itself did not induce Fas and Fas-L expression in cells not exposed to UV-B, the addition of PMA to the culture medium of the UV-B-treated cells markedly and dose-dependently enhanced the cellular expression of Fas and Fas-L. This seems to suggest that a chemical carcinogen such as PMA can facilitate the expression of Fas and Fas-L in cells injured by UV radiation. In our experiments, the expression of Fas and Fas-L preceded the fragmentation of DNA. In addition, a more pronounced fragmentation of DNA was observed in the detached than in the attached cells. These results indicate that the cells that express Fas and Fas-L undergo DNA fragmentation and become detached from the wall of the flask. Other than the expression of mRNAs of Fas and Fas-L, the molecular changes that occur in the cell after UV-B exposure are not known.

Protooncogenes such as *p53* and c-*myc* are associated with the occurrence of apoptosis (*29-32*), while *bcl-2* inhibits apoptosis (*33, 34*). Cells express protooncogenes such as *p53*, c-*fos*, and c-*jun* in response to mild UV radiation (*2, 29*). Cells also express c-*myc* in response to PMA (*35*). Thus, numerous protooncogenes may be induced in the cells exposed to UV and PMA. The present study evaluated serial changes in the expression of mRNA for *p53*, *bcl-2*, and c-*myc*. The cells examined under various conditions, even the apoptotic cells, consistently expressed mRNAs for *p53* and *bcl-2*. In contrast, c-*myc* mRNA was detected initially, but became undetectable in the cells that abundantly expressed Fas and Fas-L, although no DNA fragmentation was observed at that time. The c-*myc* protein not only acts as a transcriptional factor but is also associated with apoptosis (*31, 32*). Cells with a high level of c-myc protein are prone to death by apoptosis (*31, 32*). Since we did not measure c-myc protein in our study, we do not know whether the cells destined to die by apoptosis contained this protein. It is possible that the G_0-G_1 fibroblasts destined to die by apoptosis do not require c-myc protein, and that c-*myc* mRNA disappears before the cleavage of DNA begins.

The dose of UV-B of 80 J/m^2 used in this study is equivalent to that reaching the surface of the skin after 320 min of exposure to the sun at noon on a sunny September day in Dallas, Texas (*36*). Thus, our experimental conditions may be clinically relevant, for instance, to the effects of sunbathing. This dose of UV-B significantly altered the L-929 cells. Because both UV and PMA are known tumor promoters (*2, 15*), it would seem that the cells exposed to both UV and PMA would be more likely to show the characteristics of cancer cells than the cells exposed to either UV or PMA alone. Instead, however, the cells pretreated with UV-B underwent apoptosis when exposed to PMA. It is generally believed that abnormal cells that can potentially lead to defective cellular offspring are programmed to die by apoptosis (*23, 37*). Our results support this theory.

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