Psychological Stress and Phorbol Ester Inhibition of Radiation-Induced Apoptosis in Human Peripheral Blood Leukocytes

L. David Tomei, Janice K. Kiecolt-Glaser, Susan Kennedy, and Ronald Glaser

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Abstract. Apoptosis is a process of genetically programmed alterations of cell structure that lead to failure of proliferation and differentiation, and eventual cell death. Apoptosis is induced by a variety of toxic insults including growth factor deprivation and ionizing radiation. This process may function to protect against the appearance of heritable phenotypic changes in cells and may be a critical factor in normal cellular immune function. Phorbol esters inhibit apoptosis, but little is known about factors that regulate this process physiologically. In this study, we demonstrate an association between an acute psychological stressor, taking examinations, and the induction of substantial and reversible changes in the response of peripheral blood leukocytes to gamma irradiation and to phorbol ester treatment. These data suggest that psychological stress may induce physiological changes that regulate the ability of immune cells to initiate apoptosis.

Key Words. Phorbol esters, apoptosis, radiation, cell death, DNA, human leukocytes, academic stress.

The mechanisms underlying the interaction between the central nervous system (CNS) and the immune system are an area of intense study. It is generally thought that hormones are key mediators of many of these interactions (Hall and Goldstein, 1981; Hall et al., 1982). For example, it is known that glucocorticoids have immunoregulatory activities across a broad spectrum of the cellular immune response (Munck et al., 1984). In addition, there is now evidence to suggest that there is actually “hard wiring” between the CNS and the immune system as demonstrated by the observation that nerve endings are in direct contact with T-lymphocytes in the spleens of rats (Felten et al., 1987; Felten and Olschowka, 1987).

The communication between the CNS and immune systems is not unidirectional, but is functionally a loop with feedback from the immune system to the CNS. For example, interleukin-1 (IL-1), produced by macrophages, has the ability to affect

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hypothalamic activity promoting corticotropin releasing factor (CRF) secretion and adrenocorticotropic hormone (ACTH) stimulation (Sapolsky et al., 1987), which in turn stimulates the production and secretion of cortisol (Gillis et al., 1979; Snyder and Umane, 1982; Kelso and Munck, 1984; Malarkey and Zvara, 1989). Additional evidence for this feedback loop is provided by the finding that antigenic challenge results in an increase in the firing rate of neurons located in the hypothalamus (Besedovsky et al., 1977) and that lymphocytes produce ACTH and endorphin-like peptides. This lymphocyte-derived ACTH may be similar to pituitary-derived ACTH (Bialock and Smith, 1980, 1981; Smith and Bialock, 1981).

Since the original description by Kerr et al. (1972), several reports have suggested that apoptotic cell death is closely linked to normal immune function and may be critical to the underlying mechanisms of cell-mediated death of target cells (Duvalli et al., 1985; Ucker, 1987; Wyllie, 1988). Recently, there has occurred a dramatic rise in the number of reports in the literature on programmed cell death, reflecting an increased awareness of the importance of how cells die (Wyllie, 1988). Our laboratory and others have reported evidence that the death of eukaryotic cell following various modes of cytotoxic stress, including ionizing radiation and glucocorticoid treatment, is a consequence of a new pattern of specific gene expression that leads to endonucleolytic degradation of genomic DNA (Williams et al., 1974; Seaman et al., 1981; Umanovsky et al., 1981). We recently reported that low concentrations of tumor-promoting phorbol esters specifically block apoptosis in vitro induced by either growth factor deprivation or ionizing radiation (Kanter et al., 1982, 1984; Tomei et al., 1986, 1988). The observation that apoptosis can be inhibited by specific agents demonstrated that cell death can be modulated, which is of special interest since this introduces the possibility of therapeutic intervention in several diseases such as cancer (Szende et al., 1989, in press).

Psychological distress has been associated with down-regulation of the cellular immune response (Ader, 1981; Keller et al., 1981; Coe et al., 1988). Similar changes in the immune response were observed in a series of studies from our laboratory using peripheral blood leukocytes (PBLs) obtained from medical students during a 3-day examination block, compared to PBLs obtained at a low-stress baseline 1 month previously. These changes include decrements in natural killer (NK) cell activity (Kiecolt-Glaser et al., 1984; Glaser et al., 1986), lower mitogenic responsiveness (Glaser et al., 1985), marked suppression of gamma interferon production by lymphocytes stimulated with concanavalin A (Con A), reactivation of latent Epstein-Barr virus (EBV) as measured by increases in EBV-specific antibody levels, a decrease in cell killing by memory T-lymphocytes of EBV-transformed autologous B-lymphocytes, and increases in both plasma and intracellular cyclic AMP levels in PBLs (Glaser et al., 1986, 1987). In addition, an impairment of DNA repair in PBLs obtained from highly distressed unmedicated psychiatric inpatients, as compared to PBLs from blood bank controls, was found after exposing the PBLs to ionizing radiation (Kiecolt-Glaser et al., 1985).

Using the same paradigm to study the effects of academic stress on phorbol ester inhibition of radiation-induced apoptosis in PBLs, we report here the first evidence that lymphocyte death in humans may be modulated by an acute psychological stressor. These experimental results demonstrate a radical alteration in the response of freshly obtained PBLs to lethal levels of gamma irradiation when combined with phorbol ester treatment. The data demonstrate that 12-O-tetradecanoylphorbol-13-acetate (TPA) blocks lethal radiation-induced DNA fragmentation and that such activity is profoundly affected by physiological changes associated with an acute psychological stressor in humans.

Methods

Subjects and Timing of Samples. Blood samples were obtained from 14 healthy, nonmedicated first-year medical students attending The Ohio State University College of Medicine (mean age = 23, 9 males and 5 females). Samples were obtained between 1200h and 1300h at two points in each of two studies. In Study 1, total cellular DNA in the PBLs was measured. Samples were obtained 2 weeks after the start of the academic year in September (Baseline A) and 1 month later in October during the students' first series of academic examinations (Examination A).

In Study 2, DNA synthesis was measured to further confirm changes in total cellular DNA content observed in Study 1. These samples were obtained in January, shortly after students returned from Christmas vacation (Baseline B) and finally in February during the second series of academic examinations (Examination B). Four of the original 14 students did not return for the last sample (Examination B) so there were 10 subjects for the latter study.

Self-Report Data. The Brief Symptom Inventory (BSI; Derogatis and Spencer, 1982) provided an independent assessment of the relative degree of distress associated with each of three sample points. Subjects rated each of the 53 items on the basis of the amount of associated discomfort during the past week. The Anxiety scale was used because it is the most responsive scale for short-term distress associated with examinations (Kiecolt-Glaser et al., 1984).

Briefly, in this test students were asked a number of health behavior questions each time blood was drawn. They were asked to indicate the total number of hours of sleep they had in the last 3 days, as well as any change in weight in the last week. In addition, they were asked to indicate the number of alcoholic drinks they had consumed within the last 48 hours, and within the last week. They were also asked to list all medications taken within the last week, whether prescription or nonprescription.

Treatment of Cells. The PBLs were separated from whole blood on Hypaque-Ficoll gradients and washed twice with phosphate buffered saline as previously described (Glaser et al., 1987). Each sample of PBLs was treated with 10 μg/ml phytohemagglutinin (PHA) in RPMI 1640 medium supplement with 10% heat-inactivated fetal bovine serum (FBS) for 24 hours at 37°C. The cell samples were then divided into four 2 ml aliquots, each containing 2.5 × 10^6 cells. The appropriate groups were irradiated (10 Gy in a 137Cs irradiator) either in normal medium or medium containing TPA (10^-4M). The cells were then incubated for 96 hours at 37°C in RPMI 1640 medium containing 20% FBS. Finally, each sample group was divided into three aliquots, and triplicate measurements of either total DNA or titrated thymidine ([^3H]THd) incorporation were performed. Total DNA content was previously determined to be equivalent to 4 μg calf thymus DNA/5 × 10^6 cells and within the linear range of the assay (Tomei et al., 1988).

Bisbenzamide Fluorescence DNA Assay. Following the 96-hour incubation, each sample was washed twice by centrifugation, then resuspended in PBS. The DNA level in each replicate cell sample was determined based on the fluorometric method described by Kantor and Schwartz (1982) using an Aminco Bowman spectrophotometer. Ratios of duplex/single-strand DNA were also determined in each of the experimental groups by comparison of
bisbenzamide fluorescence following either partial or complete denaturation. The data are expressed as percent control of levels obtained in matched cell aliquots for each sample.

**Incorporation of ³H-dThd.** Stimulation of DNA synthesis was determined by measuring the level of ³H-dThd incorporation into acid insoluble material in replicate samples (n = 3) as previously described (Tomel et al., 1981). Since we have demonstrated that incorporation of ³H into cellular DNA can induce apoptosis (Tomel et al., 1988), the specific activity was adjusted to 2 Ci/mmol (0.5 µCi/ml). Cumulative incorporation was then measured during the 96-hour incubation period following irradiation.

**Nutritional Status.** Since the immune response can be affected by poor nutrition, we measured a plasma protein and albumin to provide one index of nutritional status (Chandra and Newberne, 1977). The procedure used to measure plasma albumin was the Bromersol Green dye-binding method of Rodkey (1965), later modified by Doumas et al. (1971). This procedure is recognized as a particularly good procedure as compared to other dye-binding techniques because of its specificity and freedom from interference.

**Statistical Analysis.** For both studies, data were first analyzed in a 4 × 2 multivariate analysis of variance (MANOVA) with two within-subjects variables, the treatment group and the change from baseline to examination. Following observation of significant main effects for both of the within-subjects variables, cell treatments and change from baseline to examination, the data from both studies were subsequently analyzed using univariate analysis of variance and appropriate post hoc tests (Waller and Duncan, 1969).

**Results**

**Behavioral and Nutritional Measures.** Behavioral data from each of the two studies were similar. For study 1, anxiety increased significantly during examinations (F = 4.86; df = 1, 13; p < 0.05). The T score mean for Baseline A was 57.67 (SEM = 2.65), whereas the mean for Examination A was 64.40 (SEM = 2.63).

Students reported significantly less sleep at Baseline A (mean = 22.27 hours, SEM = 0.58) than in the 3 days preceding Examination A (mean = 19.36 hours, SEM = 0.60) (F = 36.98; df = 1, 13; p < 0.001). However, the amount of sleep was not significantly correlated with the apoptosis data, with correlations of -0.06 for the combination TPA and radiation group, 0.06 for the group with TPA alone, and -0.06 for the group with radiation alone.

For Study 2, anxiety was again significantly higher during Examination B than the corresponding Baseline B (F = 6.61; df = 1, 9; p < 0.05). As in study 1, students reported fewer hours of sleep in the 3 days before Examination B than in the period before Baseline B; however, the amount of sleep again was not significantly correlated with the apoptosis data.

**Total Cellular DNA Content.** Normal human PBLs exposed to 0.1 to 9 Gy gamma radiation were observed to undergo extensive DNA strand breakage when measured at 48 hours post-irradiation using the procedure originally described by Kanter and Schwartz (1982). The maximum degree of DNA fragmentation was found to occur approximately 96 hours post-irradiation in accord with data previously reported (Tomel et al., 1988). Therefore, for these studies the measurement of cellular DNA was performed at a single time point, 96 hours post-irradiation. A dose of 10 Gy (¹³⁷Cs, 1.5 Gy/min) was used since this level of radiation resulted in approximately 90%-95% suppression of ³H-dThd incorporation over 96 hours post-irradiation and a loss of 25-35% in viable cells (data not shown).

Since the cellular response kinetics are considerably more complex than studies of this nature can reveal, we defined and controlled two critical parameters: (1) treatment of cells was initiated after 24 hours of PHA stimulation; (2) a fixed 96-hour response period was used to define responses in terms of total cellular DNA and DNA synthesis. We considered the process of the PHA mitogenic response to be at or near completion at the time of TPA treatment and/or irradiation. Since no significant difference was observed in the duplex/single DNA strand ratio between any of the four experimental treatment groups 96 hours post-irradiation (p > 0.99) (Kanter and Schwartz, 1982), we also considered that apoptosis was at or near completion at the time of the final assay. Therefore, any difference in total DNA content between treatment groups was considered to reflect a difference in total cellular duplex DNA.

Total cellular DNA content was measured in PHA activated PBLs obtained at Baseline A and Examination A (Fig. 1). The MANOVA showed a significant main effect for differences among cell treatment groups (F = 59.87; df = 1, 13; p < 0.001).

**Fig. 1. Total DNA content in peripheral blood leukocytes (PBLs) obtained from individual subjects at Baseline A (triangles) and Examination A (circles) periods**

Each point represents the mean of triplicate measurements of aliquots of PBLs. The values are expressed as % of the matched untreated control PBL aliquot (Radiation + TPA) 10 Gy gamma irradiation followed immediately by addition of 10⁻⁴M TPA (Radiation), irradiation alone, and (TPA) addition of 10⁻⁴M TPA alone. TPA = 12-O-tetradecanoyl-phorbol-13-acetate.
Planned within-subject comparisons between Baseline A and Examination A samples (Table 1) showed significant increases for TPA alone, and for TPA combined with radiation, whereas radiation alone did not exhibit a significant change. PHA-stimulated PBLs treated with TPA alone showed an increase in DNA content over control values at Baseline A.

**Table 1. Effects of acute psychological stress on total cellular DNA in PBLs following gamma radiation and treatment with TPA**

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Baseline sample (A)</th>
<th>Examination sample (A)</th>
<th>Baseline sample (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>2. TPA (10^4 M)</td>
<td>120.92 ± 3.75</td>
<td>181.55 ± 14.21</td>
<td>158.49 ± 40.90</td>
</tr>
<tr>
<td>3. Irradiation (10 Gy)</td>
<td>74.31 ± 2.80</td>
<td>70.96 ± 10.87</td>
<td>31.15 ± 5.64</td>
</tr>
<tr>
<td>4. TPA + irradiation</td>
<td>77.44 ± 2.95</td>
<td>118.42 ± 16.14</td>
<td>23.72 ± 6.30</td>
</tr>
</tbody>
</table>

Note: PBL = peripheral blood leukocyte. TPA = 12-0-tetradecanoylphorbol-13-acetate. PHA = phytohemagglutinin. FBS = fetal bovine serum.

1. Cell cultures: Each sample of PBLs was treated with 10 μg/ml PHA in RPMI 1640 medium supplemented with 10% FBS for 24 hours at 37°C. The appropriate groups were irradiated (10 Gy in a 60Co irradiator) with and without TPA (10^4 M) as described in Methods.
2. Nonsignificant change over sample points.
3. p < 0.001 for change over sample points using a repeated measures analysis of variance.
4. Means with different superscripts within each row differ at p < 0.01.

Although TPA is known to induce blastogenesis (Clouse et al., 1984), it was not clear whether the increased DNA content reflected an increase in cellular DNA synthesis or an inhibition of DNA degradation associated with apoptosis. The effect of TPA on DNA content in irradiated PBLs obtained in the Baseline A PBL samples indicated that TPA had no significant effect on the reduction of cellular DNA content by radiation as compared with cells receiving radiation alone. In the Examination A blood samples, PBLs exhibited an increased level of total cellular DNA following TPA treatment alone, an increase which was significantly greater than that observed in PBLs obtained during the Baseline period. However, irradiation of the cells produced the same loss in DNA in both Baseline and Examination PBL samples. These data indicate that although radiation lethality had not significantly changed, the response of the TPA-treated PBLs to irradiation was markedly different.

Results obtained with PBLs from the Examination A blood sample indicate that PBLs from a significant number of subjects actually showed increased DNA levels with a mean of 118.4% (p < 0.01) of the level measured in matched untreated cell aliquots. This observation suggests that treatment of PBLs with TPA immediately after irradiation resulted in the prevention of the loss of cellular DNA in cells that were obtained from the medical students under conditions of acute psychological stress.

**DNA Synthesis.** Since TPA treatment alone increased total cellular DNA levels in irradiated PBLs obtained in the Examination A blood sample and previously reported experiments using C3H-10T1/2 cells (Tomei et al., 1988) revealed that TPA blockade of radiation-induced apoptosis was associated with concomitant cell cycle activation, we wished to determine whether the increase in total cellular DNA content could be associated with an increase in DNA synthesis as measured by 3H-dTdR incorporation into acid insoluble material.

As shown in Table 2 and Fig. 2, in PBLs obtained at Baseline B, treatment of PBLs with TPA alone decreased 3H-dTdR incorporation by 16.16% compared with the control aliquot which received PHA alone. In this same sample, radiation alone produced an 81.29% suppression of 3H-dTdR incorporation, and combining TPA treatment with radiation did not significantly change the PBL response.

**Table 2. Effect of acute psychological stress on 3H-dTdR incorporation into cellular DNA of PBLs following gamma irradiation and treatment with TPA**

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Baseline sample (A)</th>
<th>Examination sample (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>15,793</td>
<td>100.00</td>
</tr>
<tr>
<td>2. TPA (10^4 M)</td>
<td>13,242 ± 1810</td>
<td>83.84 ± 11.47</td>
</tr>
<tr>
<td>3. Irradiation (10 Gy)</td>
<td>2,954 ± 276</td>
<td>18.71 ± 1.75</td>
</tr>
<tr>
<td>4. TPA + irradiation</td>
<td>2,830 ± 525</td>
<td>17.92 ± 3.31</td>
</tr>
</tbody>
</table>

**Note:** PBL = peripheral blood leukocyte. TPA = 12-0-tetradecanoylphorbol-13-acetate. cpm = counts per minute. 3H-dTdR = thymidine.

1. Immediately following treatment of PBLs, 3H-dTdR was added to each aliquot (1 μCi/ml, 5 Ci/mM). Cumulative incorporation of 3H was measured in the acid insoluble material by liquid scintillation counting techniques. Mean cpm/ sample was then calculated (n = 3) and expressed as % control response.
2. Samples differ significantly, F = 13.70; df = 1, 13; p < 0.01.
3. Nonsignificant difference, F = 2.82; df = 1, 13.
4. Samples differ significantly, F = 4.01; df = 1, 13; p < 0.0001.

In the Examination B PBL samples, radiation alone resulted in a marked suppression of DNA synthesis similar to that observed in the Baseline B sample. However, the response to TPA alone revealed increased DNA levels to 333.62% of the PHA control levels. Furthermore, in contrast to the Baseline B sample, TPA treatment of the irradiated PBLs produced a substantial increase in 3H-dTdR incorporation to 241.97% of PHA control levels. Contrary to expectations, the TPA stimulation of DNA synthesis in PBLs was not significantly different from the response of the irradiated aliquots. The results obtained with PBLs obtained from Baseline B and Examination B blood samples suggest that TPA inhibits radiation-induced cell death in the PBLs obtained from psychologically stressed normal subjects. The treatment of these cells with TPA may also induce a substantial and concomitant increase in DNA synthesis in cells that have already sustained radiation-induced damage, damage that would otherwise be lethal.

**Discussion**

As we previously reported, cell death following ionizing radiation may not be due directly to the production of molecular damage (Tomei et al., 1988) since subsequent
cell death can be blocked with no evidence that the direct radiation-induced molecular damage is lessened. We also reported that phorbol esters, dihydro-teledocin-B, and epidermal growth factor blocked apoptosis that was induced by several modes of toxic insults including growth factor deprivation, $^3$H-dTThd incorporation into cellular DNA, and gamma irradiation (Kanter et al., 1984; Tomei et al., 1988). Inhibition of apoptosis could then be expected to increase mutation frequencies and transformation rates following DNA damage by radiation or chemicals and data supporting such a prediction has been in the literature for several years (see Fisher et al., 1981). Therefore, modulation of the expression of apoptosis in general through pharmacological or physiological means can be expected to have a profound influence on cell and tissue function and will affect the interpretation of experimental data (Tomei and Cope, in press).

These data have broad implications regarding the need to define and control critical parameters that directly influence expression of cellular apoptosis in human studies. It is increasingly evident that cytotoxic insults that result from virus infection, chemical exposure, or ionizing radiation may elicit expression of gene directed cell death marked by the appearance of extensive and characteristic fragmentation of cellular DNA to 200 base pair units. In instances where toxic stress is part of a therapeutic rationale, it is readily apparent that modulation of the ability of normal and malignant cells to initiate apoptosis may be critical to the efficacy of the antitumor therapy. Szende et al. (in press) present compelling evidence that the antitumor effects of analogs of somatostatin and luteinizing hormone releasing hormone are a consequence of their effect on control of apoptosis. These actions may involve the restoration of the ability of the tumor cells to initiate apoptosis.

Psychological stress in humans has been shown to modulate the cellular immune response and could have implications for risk of infectious disease (Glaser et al., 1987). In this study, we undertook an unconventional experimental approach designed to determine whether physiological changes associated with psychological stress could influence the ability of PBLs to initiate apoptosis following the defined cytotoxic insult of gamma irradiation.

We observed that TPA increased the total cellular DNA levels in PBLs obtained from medical students at both Baseline and Examination samples (Table 1). However, TPA did not increase $^3$H-dTThd incorporation in PBLs from the Baseline B blood samples, but rather decreased it to 82.1% of control. It is possible that this is due to TPA inhibition of cell death which could be associated with the physiological trauma of isolation and in vitro manipulation of the PBLs. If this were the case, then TPA would appear to have increased total cellular DNA levels as compared to the controls, and no increase in DNA synthesis would have been found. However, increases in cellular DNA in TPA-treated PBLs from the Examination A blood sample were found to correspond to marked increases in $^3$H-dTThd incorporation when this was examined in the Examination B PBL samples. These data were not related to OKT/3 T lymphocytes since no significant change in this population was observed in this study. Therefore, the conclusion drawn from these observations is limited but critical: these cells have presumably sustained lethal radiation damage yet are capable of both surviving as well as initiating active DNA synthesis.

On the basis of our previously reported findings that TPA can both increase the number of cycling cells (i.e., mitogenesis) while simultaneously decreasing the rate of G1/S phase transition (Tomei et al., 1981), the cell cycle kinetics involving PHA and TPA stimulation can be considered to be too complex to be controlled for in our current experimental design. Therefore, the interpretation of these results should be limited to the effect of an acute cyclical stressor, TPA, and radiation on cell survival. Further investigations will need to be performed to extend these findings to include cell cycle kinetics and growth control.

Previous work from our laboratory has demonstrated a relationship between psychological stress and DNA repair in human PBLs and rat splenic lymphocytes (Glaser et al., 1985; Kiecolt-Glaser et al., 1985). The data obtained in this study provide additional evidence for understanding how psychological distress could contribute to increased cancer risk through the modification of cell responses to environmental factors such as tumor promoters and oncogenic viruses (Tomei et al., 1987); however, the association between stress and increased cancer risk is still equivocal. These physiological changes could operate independently and/or in conjunction with stress-induced immune suppression observed by our laboratory and others (Ader, 1981; Keller et al., 1981; Kiecolt-Glaser et al., 1984; Shavit et al., 1984; Glaser et al., 1985, 1986, 1987; Coe et al., 1988). Data from rodent and human studies have shown stress-related decrements in NK cell activity, which are thought to be of particular importance because of the association of NK cells with a tumor

Fig. 2. DNA synthesis as measured by cumulative tritiated thymidine incorporation over a 96-hour posttreatment

The data are expressed as % of the matched untreated control PBL aliquot at Baseline B (triangles) and Examination B (circles) periods: (Radiation + TPA) 10 Gy gamma irradiation followed immediately by addition of 10$^{-9}$M TPA (Radiation), irradiation alone, and (TPA) addition of 10$^{-9}$M TPA alone. PBL = peripheral blood leukocyte. TPA = 12-O-tetradecanoyl-phorbol-13-acetate. $^3$H-dTThd = tritiated thymidine.
surveillance function (reviewed in Whiteside and Herberman, 1989). This is especially pertinent since it has been demonstrated recently that target cell death requires gene expression and initiation of apoptosis (Ucker, 1987).

Modulation of the ability of cells to initiate apoptosis has not been directly studied previously. Recently, Valerie et al. (1988) demonstrated that radiation-induced cell suicide results in a simultaneous increase in the replication of human immunodeficiency virus (HIV). This is consistent with other recent evidence supporting the concept that certain viruses may be capable of modulating the ability of cells to respond to cytotoxic stress through initiation of suicide (Rasheed et al., 1986; Geelen et al., 1988). It is noteworthy that Klein et al. (1987) have reported that a nonviral plasma fraction from patients with AIDS or AIDS-related complex markedly enhances cortisol-induced lymphocyte death, a process involving apoptosis. Our data support the concept that psychological stress may impair the function of the immune system through alterations in the ability of cells to initiate apoptosis, an important immunological process. It is likely that this is mediated by stress-related changes in endocrine function since glucocorticoids are effective inducers of apoptosis in lymphocytes (Duke et al., 1983).

The ability of cells to initiate apoptosis is undoubtedly the product of complex physiological interactions only recently recognized as important. The recent observations of Ucker (1987) and Penit and Ezine (1989) have revealed the possibility that modulation of apoptosis within the thymus and bone marrow may provide for a dynamic control over T-lymphocyte subpopulations in the circulation during a specific period.

The data suggest that the physiological changes that occur under acute psychological stress can lead to physiological changes and modulation of the ability of human PBLs to die following low levels of irradiation. We must assume that the TPA requirement for demonstrating a change in the ability of a cell to die reflects an important change in the cell physiology since TPA is known to act through binding to protein kinase C, which leads to altered control of a number of critical pathways in much the same manner as, for example, epidermal growth factor. How TPA and growth factors interfere with the control of proliferation, differentiation, and cell death is not known, but it is likely that the changes in the TPA response shown here reflect parallel changes in physiological responses to endogenous regulatory factors such as glucocorticoids. Nonetheless, the profound effect of academic stress on the response of irradiated PBLs to TPA represents the first experimental evidence of stress modulation of apoptosis in lymphocytes. In view of the findings that β-adrenergic receptors are modulated by glucocorticoids at the level of gene transcription, these data present another aspect of the interactions among the CNS, the endocrine system, and the immune system.

Control of the expression of apoptosis is critical to the function of several cell types including the target cells of cytotoxic effector cells. Therefore, the inhibition of the expression of apoptosis could result in suppression of immune function. If these interpretations are correct, then psychological distress could ultimately lead to progressive accumulation of errors within the cell genome, reduce immune competence, and lead to increased risk of environmentally associated malignant and infectious diseases.

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