Acta Biochimica Polonica

Vol. 47 No. 2/2000

339–347

QUARTERLY

Effect of aging on UVC-induced apoptosis of rat splenocytes✪

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Received: 22 March, 2000

Key words: splenocytes, aging, apoptosis, transcription factors

UVC-induced apoptotic symptoms such as morphological changes, DNA fragmentation, Bcl-2 and Bax protein expression were examined in primary splenocyte cultures from young (3 months) and old (24 months) rats. The activities of AP-1 and CRE transcription factors in UVC-irradiated splenocytes were also assessed. At 24 h after UVC irradiation 40% of cells derived from young rats were found to be apoptotic, which was twice as much as in splenocytes from old rats. Apoptosis in cells from old rats did not give typical symptoms like a "DNA ladder" and Bcl-2 protein downregulation, in contrast to splenocytes from young rats. No AP-1 transcription factor activity was found in UVC-irradiated splenocytes from old animals and only a trace activity in splenocytes from young animals. This indicates that, UVC-induced apoptosis in rat splenocytes is practically AP-1 independent and that cells from old rats are less sensitive to UVC irradiation than splenocytes from young rats.

Apoptosis has been recognised as a normal physiological process which through active cell demise leads to removal of unwanted, mutated and damaged cells [1]. The regulated process of cell death plays a critical role during embryogenesis, in tissue homeostasis and remodelling, and particularly in shaping and maintaining the repertoire of immune system cells [2]. Insufficient apoptosis contributes to the pathogenesis of cancer, autoimmune disorders and sustained viral infection, while excessive apoptosis results in inappropriate cell loss and consequent degenerative disorders such as AIDS and Alzheimer disease [3]. The role of apoptosis in normal physiological aging is still an open question. It is believed that

✪This paper was supported by the State Committee for Scientific Research (KBN, grant No. 6P207 037 07.

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aged organisms accumulate senescent (non-proliferating) cells as a result of replicative senescence, which occurs throughout the life of multicellular organisms [4]. One could expect that senescent cells have exhausted not only their division potential but also their capacity to undergo apoptosis. Indeed, recently it has been shown that in human fibroblasts senescing *in vitro* apoptosis is not induced upon withdrawl of serum [5]. Data concerning lymphocyte propensity for apoptosis during aging is scant and controversial [6]. The crucial event in the physiology of the immune system, which is responsible for limiting clonal expansion and the accumulation of lymphocytes following an antigen challenge, is their Fas/APO-1-induced death as well as death of cytokine-deprived activated T cells [2]. Recently, UV light has been found to induce apoptosis in human lymphoid cells [7–9]. As exposure to UV radiation triggers the response known as the "mammalian UV response" which is partly characterised by AP-1 activation [10], it is interesting to know if apoptotic symptoms could be correlated with AP-1 induction in rat splenocytes after UVC treatment. Previously, we [11] and others [12] showed that the induction of the AP-1 DNA binding activity in nuclear extracts of spleen cells from old animals was significantly lower than that in extracts from young animals. Therefore, we expected to observe differences between splenocytes derived from young and old rats in the propensity to activate AP-1 and simultaneously to undergo UVC-induced apoptosis.

MATERIALS AND METHODS

Cells. Splenocytes were obtained from the spleens of healthy young (3 months) and old (24 months) Wistar albino Glaxo rats fed *ad libitum* with a standard diet. Cells were taken up in RPMI 1640 medium supplemented with 2 mM glutamine, 25 mM Hepes-buffer solution, antibiotics and 10% foetal calf serum, and maintained at 37°C in a 5% CO_2 humidified incubator.

Apoptosis measurements. Splenocytes were UVC-irradiated for 3 s at a fluency of 500 J/m^2 (254 nm) using UV Stratalinker (Stratagene) to induce apoptosis. This UVC dose appeared to be the most effective in inducing apoptosis as checked by pilot experiments. Apoptosis was monitored in several ways, i.e. by staining with Hoechst 33258 dye [13], DNA fragmentation [14] and flow cytometry analysis [15] as described earlier [9].

For Hoechst 33258 (Sigma) staining and flow cytometry analysis, cells were fixed in 70% ethanol according to Gong *et al.* [16]. Flow cytometry analysis was done after cell staining with 4′,6′-diamidino-2-phenylindole (DAPI) (1 g/ml) and sulphorhodamine (20 μg/ml) (from Molecular Probes) at 4°C. For each time point 1×10^6 cells were stained. Cells were analysed for DNA (DAPI) and protein (sulphorhodamine) content on FACS Vantage (Becton-Dickinson) using Cell-Quest software (Becton-Dickinson).

Immunocytochemistry. Cells were centrifuged on a cytospin and then fixed in 4% paraformaldehyde for 1 h for immunocytochemical staining. The cells were washed several times in phosphate buffered saline (PBS), and treated with 3% hydrogen peroxide for 15 min. Unspecific binding was blocked by cell incubation with 5% solution of normal goat serum in PBS for 30 min. Then, the lymphocytes were incubated for 1 h with one of the following primary specific polyclonal antibodies diluted 1:1000: anti-c-Fos, anti-c-Jun, anti-Bcl-2 or anti-Bax (Santa Cruz Biotechnology). Then cells were treated for 1 h with avidin-biotin complex and the colour reaction was developed using diaminobenzidine (DAB) chromogen (Sigma). Every step was preceeded by extensive washing in 0.3% solution of Triton X-100 in PBS, at room temperature.

Gel shift assay. Nuclear extracts for gel shift assays were typically obtained from 2 ×

10^7 cells as described previously [9]. The protein content of the last extract was estimated according to Bradford [17].

The double-stranded oligonucleotides harbouring the AP-1 and CRE consensus sequences were from a Stratagene "gel shift" kit (Stratagene). These oligonucleotide probes were labelled with [α-^{32}P]dCTP (Amersham) by terminal transferase (Boehringer, Mannheim) and purified on Nick-columns purchased from Pharmacia. The binding reaction was carried out in 16 μl of the mixture containing EMSA buffer (20 mM Hepes, pH 7.8, 0.2 mM EDTA, 50 mM KCl, 1 mM dithiothreitol, 10 mg/ml bovine serum albumin and 4% Ficoll), labelled probe (about 20 000 c.p.m.), 10 μg of nuclear extract, and 0.4 mg poly(dI-dC) as a non-specific competitor. A 50-fold excess (1 μl) of a cold sequence was added as a specific competitor where necessary. After 20 min incubation at room temperature, 1 μl of 0.1% bromophenol blue was added and the samples were electrophoresed for 2 h through a 4% polyacrylamide gel (30 : 1 cross-linked) at 20 mA in a cold room. Finally, the gels were dried and exposed overnight to Hyperfilm-MP (Amersham), in the presence of intensifying screens.

RESULTS

Cell morphology

Morphological observation was done after staining of UVC-irradiated lymphocyte with Hoechst dye. The typical apoptotic cells (shrunken and with apoptotic bodies) were visible in the cultures from young, as well as old, rats as early as at 4 h after UVC irradiation, although the fraction of apoptotic cells was much smaller in the population of splenocytes derived from old in comparison with young rats (Fig. 1).

Internucleosomal DNA fragmentation

Internucleosomal DNA fragmentation was already observed 2 h after UVC-irradiation in splenocytes from young rats (Fig. 2A). Only a trace of DNA fragmentation was visible in control, non-irradiated cells (Fig. 2B). Oligo-

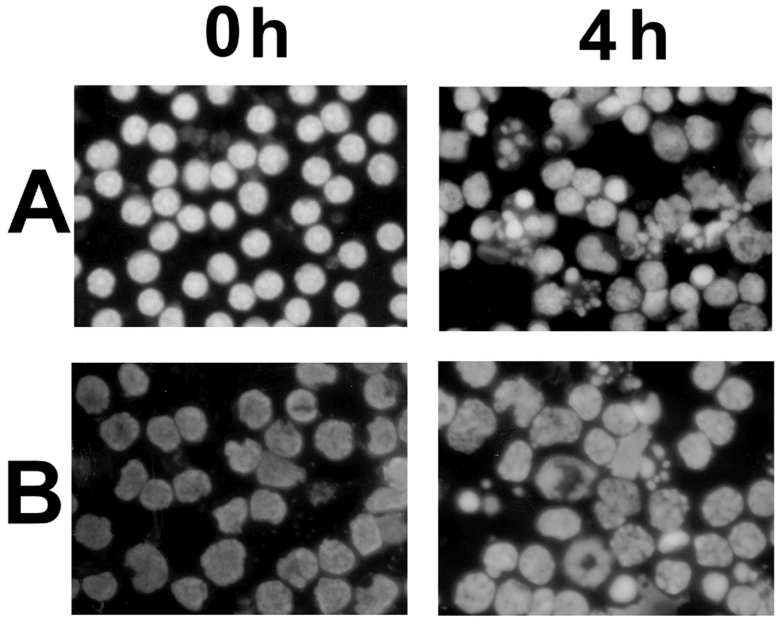

Figure 1. Morphology of splenocytes derived from young (A) and old (B) rats.

Cells were untreated (0 h) or UVC-treated and harvested 4 h after irradiation (4 h). Cells were fixed and stained with Hoechst dye as described in Material and Methods.

nucleosomal DNA fragmentation was not observed in splenocytes derived from old rats, either the UVC-irradiated (Fig. 2C) or control (Fig. 2D).

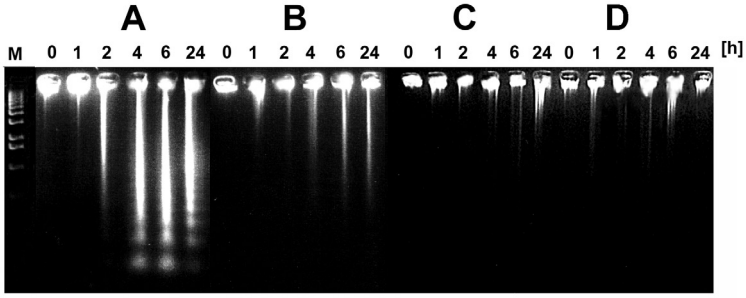

Figure 2. Agarose gel electrophoresis of DNA isolated from UVC-irradiated young (A), control young (B) UVC-irradiated senescent (C), and control senescent splenocytes (D).

Cells were collected at different times after UVC irradiation, as indicated. Lane M: molecular size marker (1 kbp from Pharmacia). The results are representative of at least three separate experiments.

(panel B, control and panel A, irradiated) and old (panel D, control and panel C, irradiated) rats. At 24 h after UVC exposure about 40% of cells from young rats were found in the sub-G_1 population. At the same time splenocytes from old rats comprised only about 20% of the cells in the sub-G_1 population. Also, the fraction of spontaneously dying cells (control) was larger in splenocytes from young than from old rats (18% and 9%, respectively) as determined after 24 h of cultivation.

Formation of a sub-G_1 population

Changes in DNA integrity were assayed accurately and reproducibly by flow cytometry analysis. In the nuclei of apoptotic cells DNA stainability was reduced as manifested by the appearance of a sub-G_1 peak on the cellular DNA content frequency histograms. Figure 3 shows typical histograms for cells from young

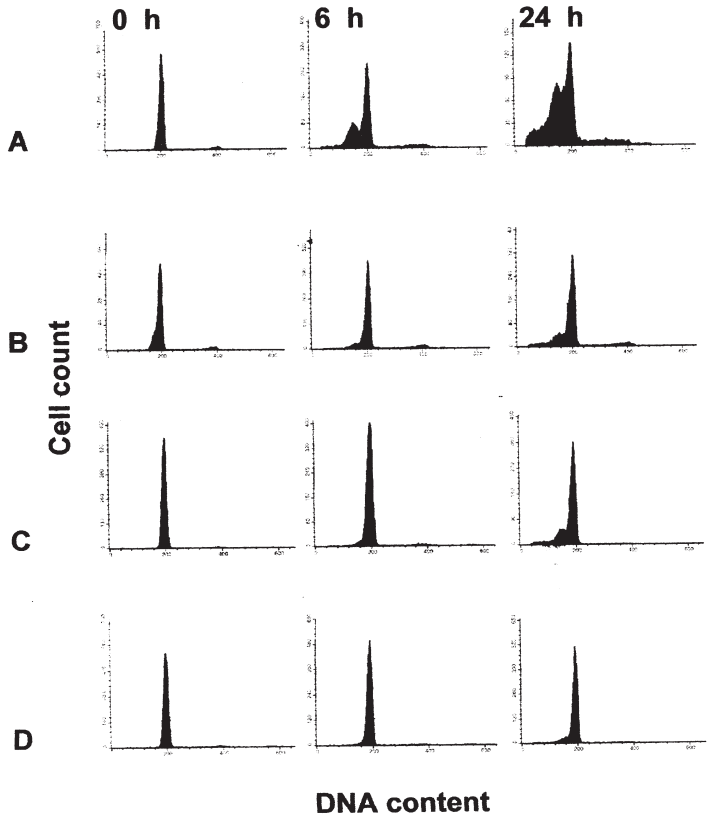

Figure 3. Flow cytometry analysis of the sub-G_1 fraction in young (A and B) and old (C and D) rat splenocytes.

Cells were untreated (B and D) or UVC-irradiated (A and C). Time after treatment is indicated. The results are representative of three

Bcl-2 and Bax protein expression

Bcl-2 protein is typically expressed in resting as well as in proliferating lymphocytes and protects them against apoptosis. As Bax acts against Bcl-2, it's increased expression is observed in cells induced to undergo apoptosis [18]. Using specific polyclonal antibodies we measured the expression of Bcl-2 and Bax proteins in splenocytes in which apoptosis was induced with UVC light. A high basal level of Bcl-2 protein was observed in cells derived from both young and old rats. At 2 h after UVC irradiation the level of Bcl-2 decreased in cells from young but not from old rats (Fig. 4). Bax expression was unchanged after irradiation both in cells from young and from old rats (not shown).

AP-1 transcription factor activity

Figure 5 shows the AP-1 and CRE transcription factor binding activities in UVC-irradiated splenocytes measured by gel shift assay. The very low AP-1 activity was detected in splenocytes from young but not from old rats. For comparison, the AP-1 activity of a nuclear extract derived from proliferating splenocytes (harvested at 4 h after Concanavalin A treatment) is shown on the same gel (Fig. 5A, lane 9). In contrast to AP-1, the activity of CRE was very high but not UVC inducible, in cells derived both from young and old rats. Expression of the protein components of AP-1, namely c-Jun and c-Fos, showed a small increase in the c-Fos level (which cannot form a homodimer) at 2 h after irradiation only in cells from young rats (not shown).

DISCUSSION

AP-1 and apoptosis

The AP-1 transcription factor, which is a dimer composed of Jun and Fos family members, has been recognised to play a role in cell differentiation, proliferation, transformation, stress response [19] and, recently, in apoptosis [20]. Although a great deal of information has been gathered about short wavelength UVC signalling through c-Jun amino terminal kinase and transcription factor AP-1 activation [21], the intracellular pathway re-

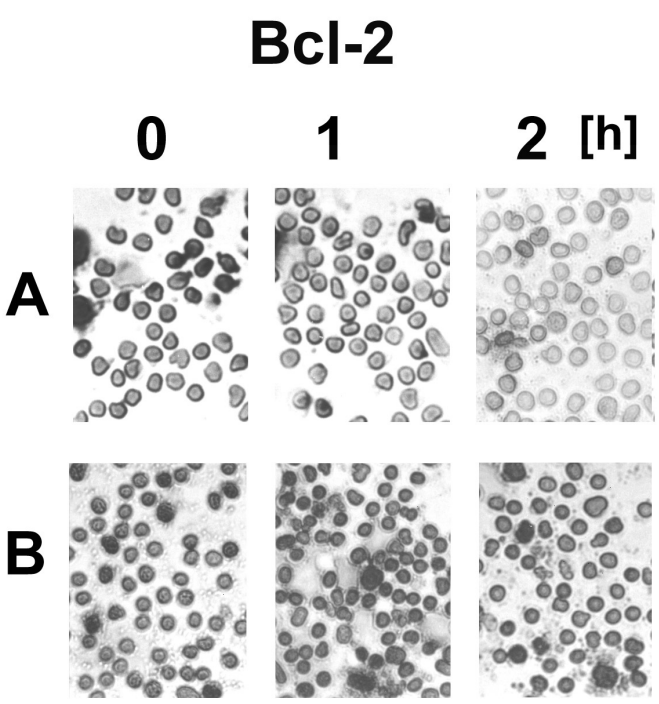

Figure 4. Immunostaining of Bcl-2 protein in young (A) and senescent lymphocytes (B), untreated (0 h), or in cells harvested 1 h and 2 h after treatment with UVC.

The results are representative of three separate experiments.

sponsible for UV-induced apoptosis has not been definitely identified. As UV induces apoptosis in lymphoid cells [7–9] and exposure to UV radiation triggers AP-1 activation [10], we wanted to know if apoptotic symptoms such as DNA fragmentation and the Bcl-2/Bax protein ratio could be correlated with AP-1 induction in rat splenocytes after UVC treatment. It has been found that 24 h after UVC irradiation about 40% of splenocytes derived from young rats were in the sub-G_1 population. Moreover, morphological changes, internucleosomal DNA fragmentation and Bcl-2 downregulation indicated that these cells underwent apoptosis. Suprisingly, induction of AP-1 in UVC irradiated splenocytes is neglible in young and hardly detectable in old rats. AP-1 induction was also not evident in the cells treated with lower dose of UVC, namely 100 J/m^2 (not shown). On the other hand, using either 100 J/m^2 or 500 J/m^2 AP-1 induction was demonstrated in human lymphocytes stimulated to proliferate with interleukin-2 [22]. Previously, we have showed that AP-1 was induced in rat thymocytes undergoing apoptosis following glucocorticoid treatment [23, 24]. AP-1 activation was also shown in several different systems of apoptotic induction (e.g. [25–31]). On the other hand, there is a growing body of evidence that neither c-*jun* nor c-*fos*, nor even both of them, are necessary for apoptosis induction [32–34]. It seems that AP-1, depending on the cell type and apoptotic inducer, is either completely not involved or acts as a protector or initiator of apoptosis [20].

Aging and apoptosis

The immune system plays a crucial role in the aging of an organism and apoptosis is critical for functioning of the immune system [6]. Accordingly, one can expect that a propensity to undergo apoptosis is one of the most important features of the aging process. Unfortunately, data concerning apoptosis of aging cells, both *in vivo* and *in vitro*, are very limited and controversial. The main observation from human lymphocytes is that the level of AICD (Activation Induced Cell Death) increases

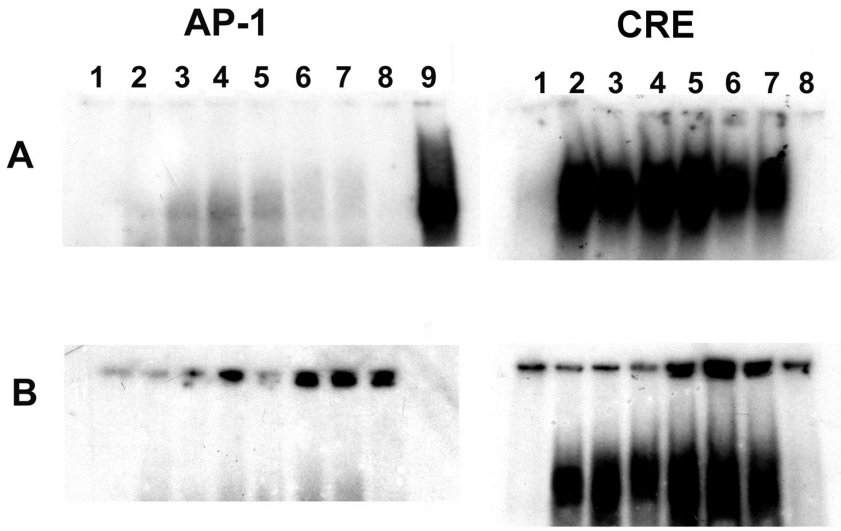

Figure 5. The time course of AP-1 and CRE transcription factor DNA-binding activities in young (A) and senescent (B) lymphocytes treated with UVC.

Nuclear proteins were isolated from cells collected at different times after treatment. Lanes: 1, probe only; 2, 0 h; 3, 0.5 h; 4, 1.0 h; 5, 2 h; 6, 4 h; 7, 6 h. Lane 8 refers to the transcription factor activity measured in the presence of a cold specific sequence added as a competitor to the sample (4 h) in a 50-fold excess with respect the radioactive one. Line 9 in panel A refers to the activity of AP-1 in young splenocytes harvested 4 h after Concanavalin A treatment. The results are representative of at least three separate experiments.

with aging [6]. On the other hand, some data supporting the concept of decreased apoptosis in old peoples' T cells compared to young people's may be found in the report of Lechner *et al.* [35]. Using rat splenocytes we demonstrated that both young and old cells are able to undergo both spontaneous and UVC induced apoptosis, although splenocytes from old animals are less prone to cell death compared to cells from young rats. Flow cytometry analysis revealed that the subpopulation of apoptotic cells is lower by a half in the population of splenocytes derived from old then from young animals. Moreover, only cells from young rats showed internucleosomal DNA fragmentation and Bcl-2 protein downregulation. Similarly, T-cell apoptosis induced by irradiation, staurosporine, anti-CD3, or heat shock was reduced by about 50% in old compared with young mice [36]. Zhou *et al.* [37] also reported higher levels of *in vitro* and *in vivo* lymphocyte apoptosis after irradiation in young compared to old mice. It was also demonstrated that Fas expression and function were decreased in T cells of old mice, which implies a diminished capacity to proliferate and to undergo apoptosis [38]. Likewise, it has been shown that activation of the Fas receptor may be involved in UV induced apoptosis [39]. Also, our preliminary results showed that UVC irradiation caused Fas receptor aggregation which was UVC dose dependent (not shown). It is tempting to hypothesise that a diminished function in the Fas receptor signalling pathway could be the reason of the reduced propensity of old lympocytes to undergo UVC-induced cell death.

To sum up, our data show that UVC-induced apoptosis in rat splenocytes is AP-1 independent and that cells derived from old animals are less sensitive to UVC irradiation than splenocytes from young animals.

The authors are deeply indebted to Dr. Malgorzata Skup (Nencki Institute of Experimental Biology, Warszawa, Poland) for important methodological remarks concerning immunostaining.

REFERENCES

1. Raff, M.C. (1992) Social controls on cell survival and cell death. *Nature* **356**, 397–400.

2. Akbar, A.N. & Salmon, M. (1997) Cellular environments and apoptosis: Tissue microenvironments control activated T-cell death. *Immunol. Today* **18**, 72–76.

3. Thompson, C.B. (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* **267**, 1456–1462.

4. Campisi, J. (1996) Replicative senescence: An old lives' tale? *Cell* **84**, 497–500.

5. Wang, E. (1995) Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. *Cancer Res.* **55**, 2284–2292.

6. Pawelec, G., Effros, R.B., Caruso, C., Remarque, E., Barnett, Y. & Solana, R. (1999) T cells and aging. *Front. Biosci.* **4**, D216–269.

7. Gottlieb, R.A., Nordberg, J., Skowronski, E. & Babior, B.M. (1996) Apoptosis induced in Jurkat cells by several agents is preceded by intracellular acidification. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 654–658.

8. Kimura, C., Zhao, Q.L., Kondo, T., Amatsu, M. & Fujiwara, Y. (1998) Mechanism of UV-induced apoptosis in human leukemia cells: Roles of Ca2+/Mg(2+)-dependent endonuclease, caspase-3, and stress-activated protein kinases. *Exp. Cell Res.* **239**, 411–422.

9. Sikora, E., Bielak-Zmijewska, A., Piwocka, K., Skierski, J. & Radziszewska, E. (1997) Inhibition of proliferation and apoptosis of human and rat T lymphocytes by curcumin, a curry pigment. *Biochem. Pharmacol.* **54,** 899–907.

10. Schreiber, M., Baumann, B., Cotten, M., Angel, P. & Wagner, E.F. (1995) Fos is an essen-

tial component of the mammalian UV response. *EMBO J.* **14**, 5338–5349.

11. Sikora, E., Kaminska, B., Radziszewska, E. & Kaczmarek, L. (1992) Loss of transcription factor AP-1 DNA binding activity during lymphocyte aging *in vivo*. *FEBS Lett.* **312**, 179–182.

12. Pahlavani, M.A., Harris, M.D., Moore, S.A. & Richardson, A. (1996) Expression of heat shock protein 70 in rat spleen lymphocytes is affected by age but not by food restriction. *J. Nutr.* **126**, 2069–2075.

13. Darzynkiewicz, Z., Li, X. & Gong, J. (1994) Assays of cell viability: Discrimination of cells dying by apoptosis. *Meth. Cell. Biol.* **41**, 15–38.

14. Smith, C.A., Williams, G.T., Kingston, R., Jenkinson, E.J. & Owen, J.J. (1989) Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature* **337**, 181–184.

15. Del Bino, G., Skierski, J.S. & Darzynkiewicz, Z. (1991) The concentration-dependent diversity of effects of DNA topoisomerase I and II inhibitors on the cell cycle of HL-60 cells. *Exp. Cell. Res.* **195**, 485–491.

16. Gong, J., Traganos, F. & Darzynkiewicz, Z. (1994) A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. *Anal. Biochem.* **218**, 314–319.

17. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

18. Cory, S. (1995) Regulation of lymphocyte survival by the *bcl-2* gene family. *Annu. Rev. Immunol.* **13**, 513–543.

19. Angel, P. & Karin, M. (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim. Biophys. Acta* **1072**, 129–157.

20. Karin, M., Liu, Z. & Zandi, E. (1997) AP-1 function and regulation. *Curr. Opin. Cell Biol.* **9**, 240–246.

21. Tyrrell, R.M. (1996) Activation of mammalian gene expression by the UV component of sunlight – from models to reality. *Bioessays* **18**, 139–148.

22. Radziszewska, E., Piwocka, K., Skierski, J. & Sikora, E. (1999) UVC-induced death in IL-2 dependent human lymphocytes. *Cell Biol. Intern.* **23**, 97–103.

23. Sikora, E., Grassilli, E., Bellesia, E., Troiano, L. & Franceschi, C. (1993) Studies of the relationship between cell proliferation and cell death. III. AP-1 DNA-binding activity during concanavalin A-induced proliferation or dexamethasone-induced apoptosis of rat thymocytes. *Biochem. Biophys. Res. Commun.* **192**, 386–391.

24. Sikora, E., Rossini, G.P., Grassilli, E., Bellesia, E., Salomoni, P. & Franceschi, C. (1996) Interference between DNA binding activities of AP-1 and GR transcription factors in rat thymocytes undergoing dexamethasone-induced apoptosis. *Acta Biochim. Polon.* **43**, 721–731.

25. Bossy-Wetzel, E., Bakiri, L. & Yaniv, M. (1997) Induction of apoptosis by the transcription factor c-Jun. *EMBO J.* **16**, 1695–1709.

26. Kaminska, B., Filipkowski, R.K., Zurkowska, G., Lason, W., Przewlocki, R. & Kaczmarek, L. (1994) Dynamic changes in the composition of the AP-1 transcription factor DNA-binding activity in rat brain following kainate-induced seizures and cell death. *Eur. J. Neurosci.* **6**, 1558–1566.

27. Kim, R. & Beck, W.T. (1994) Differences between drug-sensitive and -resistant human leukemic CEM cells in c-jun expression, AP-1 DNA-binding activity, and formation of Jun/Fos family dimers, and their association with internucleosomal DNA ladders after treatment with VM-26. *Cancer Res.* **54**, 4958–4966.

28. Marti, A., Jehn, B., Costello, E., Keon, N., Ke, G., Martin, F. & Jaggi, R. (1994) Protein kinase A and AP-1 (c-Fos/JunD) are induced during apoptosis of mouse mammary epithelial cells. *Oncogene* **9**, 1213–1223.

29. Mollinedo, F., Gajate, C. & Modolell, M. (1994) The ether lipid 1-octadecyl-2-methyl-*rac*-glycero-3-phosphocholine induces expression of *fos* and jun proto-oncogenes and activates AP-1 transcription factor in human leukaemic cells. *Biochem. J.* **302**, 325–329.

30. Tong, L. & Perez-Polo, J.R. (1995) Transcription factor DNA binding activity in PC12 cells undergoing apoptosis after glucose deprivation. *Neurosci. Lett.* **191**, 137–140.

31. Sawai, H., Okazaki, T., Yamamoto, H., Okano, H., Takeda, Y., Tashima, M., Sawada, H., Okuma, M., Ishikura, H., Umehara, H., *et al.* (1995) Requirement of AP-1 for ceramide-induced apoptosis in human leukemia HL-60 cells. *J. Biol. Chem.* **270**, 27326–27331.

32. Gajate, C., Alonso, M.T., Schimmang, T. & Mollinedo, F. (1996) C-Fos is not essential for apoptosis. *Biochem. Biophys. Res. Commun.* **218**, 267–272.

33. Lenczowski, J.M., Dominguez, L., Eder, A.M., King, L.B., Zacharchuk, C.M. & Ashwell, J.D. (1997) Lack of a role for Jun kinase and AP-1 in Fas-induced apoptosis. *Mol. Cell Biol.* **17**, 170–181.

34. Roffler-Tarlov, S., Brown, J.J., Tarlov, E., Stolarov, J., Chapman, D.L., Alexiou, M. & Papaioannou, V.E. (1996) Programmed cell death in the absence of c-Fos and c-Jun. *Development* **122**, 1–9.

35. Lechner, H., Amort, M., Steger, M.M., Maczek, C. & Grubeck-Loebenstein, B. (1996) Regulation of CD95 (APO-1) expression and the induction of apoptosis in human T cells: Changes in old age. *Int. Arch. Allergy Immunol.* **110**, 238–243.

36. Spaulding, C.C., Walford, R.L. & Effros, R.B. (1997) The accumulation of non-replicative, non-functional, senescent T cells with age is avoided in calorically restricted mice by an enhancement of T cell apoptosis. *Mech. Ageing Dev.* **93**, 25–33.

37. Zhou, T., Edwards, C.K. & Mountz, J.D. (1995) Prevention of age-related T cell apoptosis defect in CD2-fas-transgenic mice *J. Exp. Med.* **182**, 129–137.

38. Rehemtulla, A., Hamilton, C.A., Chinnaiyan, A.M. & Dixit, V.M. (1997) Ultraviolet radiation-induced apoptosis is mediated by activation of CD-95 (Fas/APO-1). *J. Biol. Chem.* **272**, 25783–25786.

39. Leverkus, M., Yaar, M. & Gilchrest, B.A. (1997) Fas/Fas ligand interaction contributes to UV-induced apoptosis in human keratinocytes. *Exp. Cell Res.* **232**, 255–262.