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Letter to the Editor

Dual role of the anti-inflammatory sesquiterpene lactone: regulation of life and death by parthenolide

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Dear Editor,

Recently, the sesquiterpene lactone parthenolide, derived from Mexican Indian medicinal plants and European feverfew (*Tanacetum parthenium*), has attracted considerable attention because of its complex pharmacological actions. Parthenolide-containing herbs exert anti-inflammatory activity and are frequently used by Mexican Indians for the treatment of skin infections and infections of other organs. In popular medicine, the parthenolide-containing feverfew has been used orally or as infusion in conditions like arthritis and migraine. Investigation of the molecular basis of the anti-inflammatory activity of parthenolide revealed that it is a potent inhibitor of the proinflammatory transcription factor NF-κB.[1]

More recently, several studies demonstrated that parthenolide exerts a strong proapoptotic activity. It has been shown that parthenolide can sensitize a number of cancer cells to TNF-α-induced apoptosis via, at least in part, inhibition of TNF-α-mediated NF-κB activation.[2] It has also been shown that parthenolide reverts TRAIL-resistant breast cancer cells to undergo TRAIL-induced apoptosis on the basis of an NF-κB-independent mechanism.[3] Furthermore, it has been shown that parthenolide alone can kill various cancer cells by inducing apoptosis.[4] The proapoptotic feature of parthenolide raises the potential use of parthenolide as an antitumor agent. However, parthenolide, apart from its proapoptotic activity, may also protect cell death from apoptosis. We have previously shown that at nontoxic doses parthenolide suppresses CD95(APO-1/Fas)L (ligand) expression by blocking NF-κB binding to the CD95L promoter and, thereby, prevents CD95-mediated activation-induced-cell-death (AICD) of activated T cells.[5] Therefore, parthenolide may also have therapeutic potential as a new antiapoptotic substance blocking AICD in T cells.

Since parthenolide may either induce or protect from cell death, we asked how parthenolide determines the cell fate. To investigate this question, we chose Jurkat T cells as a model system. Previously, we have shown that at concentrations of 1–5 μM parthenolide protects from αCD3-induced apoptosis in Jurkat and normal peripheral blood T cells. However, a higher dose (10 μM) of parthenolide completely blocked cell proliferation and also led to an increase of dead cells.[5] To analyze the effect of parthenolide on T-cell death, we treated Jurkat T cells with different doses of parthenolide. In agreement with the previous study,[5] low doses (up to 5 μM) of parthenolide did not induce apoptosis in Jurkat T cells. However, treatment of the cells with higher doses (more than 10 μM) resulted in inhibition of the cell cycle (G_2/M arrest) accompanied by a dose-dependent induction of apoptotic cell death (Figure 1a). Apoptotic cell death can be induced through either the death receptor- or the mitochondria-mediated signaling pathways.[6,7] The apoptotic death in Jurkat T cells induced by parthenolide is not the result of death receptor-mediated pathways, since Jurkat T cells deficient of the adapter molecule FADD (Fas/Apo-1-associated death domain protein) are as sensitive to parthenolide as the parental cells (data not shown). Many stimuli that cause oxidative stress or trigger the release of intracellular Ca^{2+} stores are sufficient to induce apoptosis through the mitochondrial pathway.[6,8] Parthenolide does not trigger the Ca^{2+} signaling in Jurkat T cells (data not shown). However, an early study demonstrated that parthenolide can generate the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2), which led to apoptotic death in hepatocellular carcinoma cells.[4] Therefore, the redox status of Jurkat T cells treated with different concentration of parthenolide was monitored by the oxidation-sensitive fluorescent dyes, DCFDA for detecting peroxides (H_2O_2) and DHE for detecting $\cdot O_2^-$. Administration of parthenolide in doses higher than 10 μM resulted in a dose-dependent increase of $\cdot O_2^-$ levels (Figure 1b). In contrast to hepatoma cells,[4] no H_2O_2 could be detected in Jurkat T cells treated with parthenolide. The parthenolide-induced apoptosis can be completely blocked by the antioxidant *N*-acetyl-cysteine (NAC) demonstrating that ROS are the cause of cell death (Figure 1c), thus, parthenolide can induce oxidative-stress-mediated apoptosis in T cells. The question remaining is how does parthenolide protect from AICD in T cells?

Previous studies suggest that T-cell activation via T cell receptor (TCR) signaling involves ROS and ROS may act as a mediator of apoptosis via stimulation of CD95L expression.[9] Antioxidants such as NAC and vitamin E can block T-cell-activation-induced CD95L expression and consequently, prevent CD95-mediated apoptosis.[10] To investigate the molecular mechanism by which parthenolide protects T cells from AICD, we monitored the redox status of Jurkat T cells stimulated by TCR-crosslinking with αCD3 antibodies in the absence or presence of parthenolide. Activation of Jurkat T cells through TCR resulted in rapid generation of H_2O_2, but in the presence of parthenolide, the T-cell-activation-induced H_2O_2 levels were significantly reduced (Figure 1d). Similar results were obtained when Jurkat T cells were activated by PMA plus ionomycin, the stimuli used to mimic TCR stimulation (Figure 1e). Although parthenolide can suppress

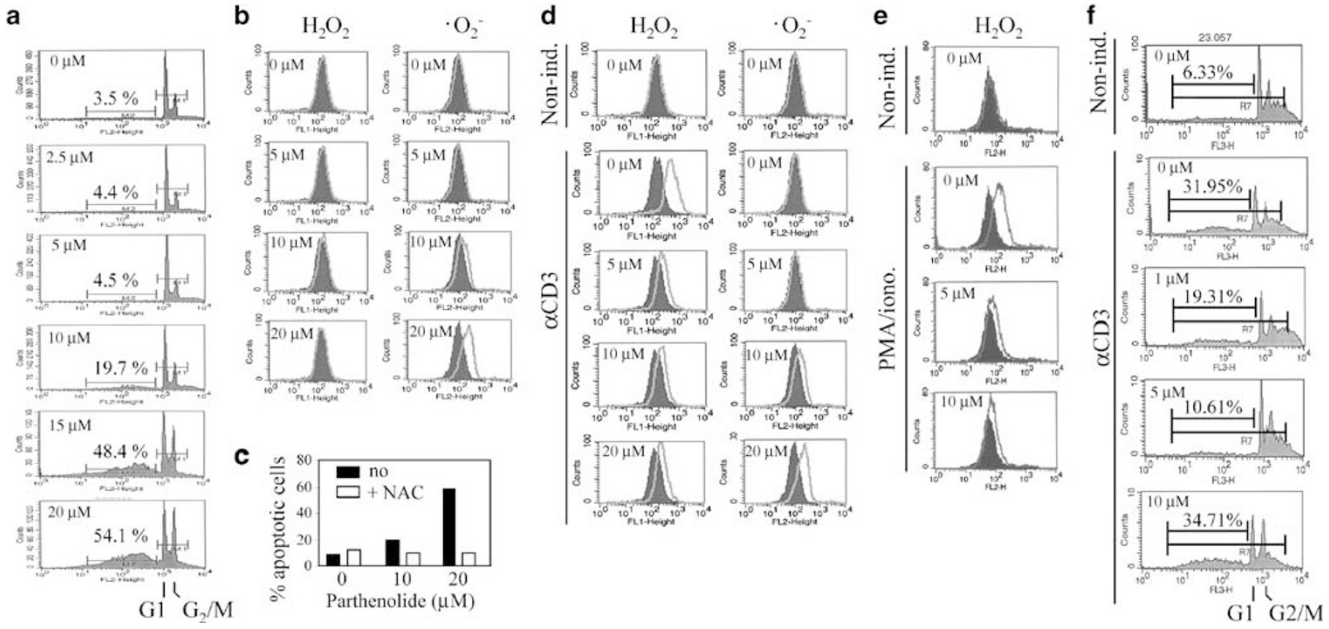

Figure 1 Effect of parthenolide on induction and protection from oxidative stress. (**a**) Dose-dependent G2/M arrest and apoptosis by parthenolide treatment in Jurkat T cells. Jurkat T cells were treated with different concentrations (0–20 μM) of parthenolide as indicated for 24 h. Apoptotic cells were analyzed by FACS for DNA fragmentation. Numbers represent percentage of apoptotic cells. (**b**) Dose-dependent generation of superoxide anions by parthenolide treatment. Jurkat T cells were treated with different doses of parthenolide as indicated. After 30 min, the redox status was monitored by the oxidation-sensitive fluorescent dyes DCFDA for H_2O_2 and DHE for O_2^-. Shifted O_2^--producing cells are indicated by the thin lines. (**c**) Parthenolide-induced apoptosis is inhibited by the antioxidant NAC. Jurkat T cells were treated with different doses of parthenolide in the absence or presence of 15 mM NAC for 24 h. Apoptotic cells were determined by FSC/SSC analysis. (**d**) At nontoxic doses, parthenolide neutralizes H_2O_2 generated by the TCR signaling pathway. Jurkat T cells were stimulated with αCD3 (plates coated with αCD3 at 50 μg/ml) in the absence or presence of different concentrations of parthenolide as indicated. After 30 min, the redox status was monitored by the oxidation-sensitive fluorescent dyes for H_2O_2 and O_2^-, accordingly. Shifted H_2O_2- and O_2^--producing cells are indicated by the thin lines. (**e**) Jurkat T cells were stimulated with PMA (10 ng/ml) and ionomycin (1 μM) in the absence or presence of different amounts of parthenolide. After 30 min, the redox status was monitored for H_2O_2 as described in (**d**). (**f**) Low but not high doses of parthenolide protect T cells from AICD. Jurkat T cells were stimulated with αCD3 in the absence or presence of different concentrations of parthenolide as indicated. After 24 h, apoptotic cells were analyzed by FACS for DNA fragmentation

T-cell-activation-induced H_2O_2 levels, at high doses, it induces $\cdot O_2^-$ and generates oxidative stress (Figure 1d). Correspondingly, low doses of parthenolide (up to 5 μM) protect Jurkat T cells from αCD3-induced apoptotic cell death, however, doses higher than 10 μM inhibit cell cycle (G_2/M arrest) and fail to protect T cell from AICD (Figure 1f).

From the above studies, we demonstrate that parthenolide, at low doses, functions as an antioxidant that can reduce the oxidative stress generated through the TCR signaling pathway. In contrast, at high doses, parthenolide by itself induces $\cdot O_2^-$ and causes oxidative-stress-mediated apoptosis. Thus, we provide an important new view on how parthenolide exerts its dual role in regulating life and death of cells. This is particularly important in applying parthenolide for therapy.

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1. Hehner SP *et al.* (1998) *J. Biol. Chem.* 273: 1288–1297
2. Zhang S *et al.* (2004) *Carcinogenesis* 25: 2191–2199
3. Nakshatri H *et al.* (2003) *Oncogene* 23: 7330–7344
4. Wen J *et al.* (2002) *J. Biol. Chem.* 277: 38954–38964
5. Li-Weber M *et al.* (2002) *Cell Death Differ.* 9: 1256–1265
6. Orrenius S *et al.* (2003) *Nat. Rev. Mol. Cell Biol.* 4: 552–565
7. Schulze-Bergkamen H and Krammer PH (2004) *Semin. Oncol.* 31: 90–119
8. Fleury C *et al.* (2002) *Biochimie* 84: 131–141
9. Devadas S *et al.* (2002) *J. Exp. Med.* 195: 59–70
10. Li-Weber M *et al.* (2002) *J. Clin. Invest.* 110: 681–690