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Regulation of Phosphatidylserine Exposure at the Cell Surface by the Serine–Base Exchange Enzyme System during CD95-Induced Apoptosis

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ABSTRACT. Early in the apoptotic process, CD95 induces a translocation of phosphatidylserine (PtdSer) from the inner to the outer leaflet of the cellular plasma membrane. In mammalian cells, PtdSer is only synthesized through a calcium-dependent exchange of the polar head group of pre-existing phospholipids, either phosphatidylcholine or phosphatidylethanolamine, by a serine. Using a pharmacological approach, we examined the influence of PtdSer synthesis on CD95-induced PtdSer exposure at the surface of Jurkat cells. We found that CD3/TCR triggering or thapsigargin treatment of Jurkat cells was accompanied both by a decreased PtdSer synthesis and by a strong reduction of CD95-induced PtdSer at the cell surface, as monitored by fluorescence-activated cell sorting (FACS) analysis of annexin V–fluorescein isothiocyanate (FITC)-labeled cells. PtdSer synthesis through the serine–base exchange enzyme system thus appeared as one of the mechanisms implicated in the recently discovered CD3/TCR-induced down-regulation of CD95-induced apoptosis. Conversely, increasing the activity of the serine–base exchange enzyme system with different drugs, either the K^+ channel blocker quinine, the cationic amphiphil stearylamine, or three different calmodulin antagonists, chlorpromazine, trifluoperazine, and *N*-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide (W7), resulted in an increased appearance of PtdSer at the surface of CD95-treated cells. Both PtdSer synthesis and CD95-induced annexin V–FITC reactivity were abrogated in ATP-depleted cells. Also, modifying the membrane potential with valinomycin (hyperpolarization) or either gramicidin or KCl (depolarization) demonstrated a strong relationship between PtdSer synthesis and annexin V–FITC reactivity in CD95-treated cells. Together, our results indicate that CD95-induced exposure of PtdSer at the cell surface could be regulated by the activity of the serine–base exchange enzyme system. BIOCHEM PHARMACOL **59**;7:855–863, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. apoptosis; CD95; phosphatidylserine; annexin V; Jurkat

Apoptosis or programmed cell death is an essential mechanism for cell removal during embryonic development and elimination of lymphocytes during thymic development. To date, a number of physiological mediators of apoptosis have been identified. Among them, CD95 (Fas) has been implicated in the apoptotic process. Cross-linking CD95 with mAbs† or with Fas ligand leads to apoptosis of numerous cells. The mechanism(s) used by Fas to trigger apoptosis involve(s) a trimerization of the Fas molecule at the cell surface followed by recruitment of FADD/MORT-1, an adaptator molecule that allows the binding of caspase-8 to the DISC [1, 2]. Following caspase-8 activation, other cysteine proteases are in turn activated, including caspase-3 and caspase-6. During CD95-induced programmed cell death (apoptosis), PtdSer, an aminophospholipid localized in the plasma membrane inner leaflet, appears in the outer leaflet [3, 4], where it participates in phagocytic recognition of the apoptotic cell [5, 6]. Externalization of PtdSer is blocked by inhibitors of caspase-8 and caspase-3 [7, 8]. Kinetic experiments have shown that the CD95 mAb, CH-11, induces a very early neosynthesis of PtdSer (detectable at 15–30 min) that precedes CD95-induced externalization of the phospholipid (evidenced at 75–90 min). Futhermore, this newly synthesized PtdSer was detectable at the cell surface in CD95-treated cells [9]. These observations prompted us to study whether PtdSer synthesis was able to regulate PtdSer exposure in cells undergoing the apoptotic process.

In mammalian cells, PtdSer is only synthesized by the serine–BEES [10, 11], located in the endoplasmic reticulum [12]. This enzymatic system is composed of two enzymes recently cloned as phosphatidylserine synthase I and II (PSS I and PSS II). PSS I synthesizes PtdSer by an exchange of the polar head group of phosphatidylcholine, while PSS II preferentially utilizes phosphatidylethanolamine as substrate [13, 14]. PtdSer synthesis is known to be Ca^{2+}- and ATP-dependent because ATP is necessary to maintain a high Ca^{2+} concentration in the endoplasmic

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† *Abbreviations:* FADD, Fas-associated death domain protein; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; CPZ, chlorpromazine; TFP, trifluoperazine; W7, *N*-(6-aminohexyl)-5-chloro-1-naphtalenesulfonamide; serine–BEES, serine–base exchange enzyme system; PtdSer, phosphatidylserine; and FACS, fluorescence-activated cell sorting.

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reticulum [15–17]. In recent years, it has been shown that the activity of the serine–BEES could be inhibited by decreasing the Ca^{2+} concentration in the endoplasmic reticulum either by triggering the CD3/T cell receptor complex in T cells [18–20] or by using the Ca^{2+}-ATPase blocker, thapsigargin, in several cell types [20–22]. Conversely, in the Jurkat T cell line, serine-BEES activity was increased by several drugs including K^+ channel blockers such as quinine [18, 23], calmodulin antagonists such as CPZ, TFP, and W7 [16, 24], and cationic amphiphils such as stearylamine [25]. In addition, PtdSer synthesis was modulated by alterations of the plasma membrane potential that modify the activity of the plasma membrane serine transporter [26].

We show here that CD95 (Fas/APO-1)-induced exposure of PtdSer at the cell surface of Jurkat cells is regulated by the amount of PtdSer synthesized through the activity of the serine–BEES. This finding confirms and extends previous reports demonstrating that CD95-induced apoptosis is down-regulated in activated T cells and T cell lines [27–30].

MATERIALS AND METHODS

Cells

Jurkat cells, either clone D we described previously [24] or clone J E6.1 (ATCC TIB 152) were cultured in RPMI 1640 (GIBCO) supplemented with 5% fetal bovine serum (Bio-Whittaker), 50 units/mL penicillin, 50 μg/mL streptomycin, 2 mM L-glutamine, and 1 mM pyruvate.

Materials

Purified CD95 mAb (immunoglobulin M, IgM, clone CH-11) and CD3 mAbs (either clone UCHT1 [immunoglobulin G1, IgG1] or X35 [immunoglobulin G2a, IgG2a]) were purchased from Immunotech. L-[3-^{3}H]serine (0.74–1.48 TBq/mmol) was from Amersham. Apoalert™ annexin V apoptosis kits (Clontech Labs) were used as described by the manufacturer. Thapsigargin, 2-deoxyglucose, and NaN_3 were purchased from Sigma, quinine and stearylamine were from Aldrich, and CPZ, TFP, and W7 were from Calbiochem.

Analysis of DNA Fragmentation

Analysis of DNA fragmentation was performed by agarose gel electrophoresis. Briefly, pelleted cells were lysed in 0.2% Triton X-100 in 10 mM Tris–HCl/1 mM EDTA, ph 7.5, and the cell lysates were treated with proteinase K and RNase, centrifuged at 15,000 *g* for 15 min. The final extracts were resolved by electrophoresis on agarose gels impregnated with ethidium bromide and visualized under UV light. Changes in DNA content of cells were also studied by cytofluorimetry with the use of the DNA marker, Hoechst 33342.

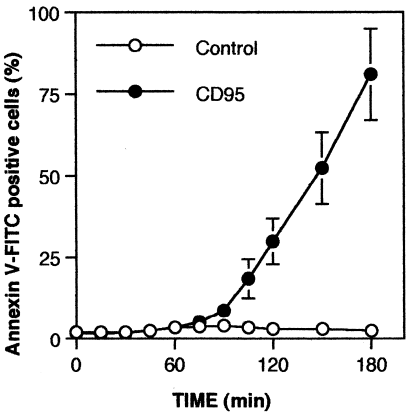

FIG. 1. Kinetics of phosphatidylserine (PtdSer) exposure at the surface of Jurkat cells treated at time 0 with 0.5 μg CD95 mAb, CH-11. Exposure of PtdSer was monitored with annexin V–FITC and FACS analysis. The results are expressed as means ± SD (N = 3).

Cytofluorimetry

Cell analysis by cytofluorimetry was performed with a FACStar plus cell sorter (Becton Dickinson).

Phosphatidylserine Synthesis

Jurkat cells (2×10^6) were maintained in 0.5 mL of a buffer (pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 2.5 mM glucose, 20 mM HEPES, 1 mM $MgCl_2$, 1 mM $CaCl_2$ at 37° in the presence of 4 μCi of L-[3-^{3}H]serine and effectors (see concentrations in the figure legends). After an incubation period of 1 hr, the cells were rapidly sedimented in an Eppendorf centrifuge and the supernatants discarded. The cellular phospholipids were extracted using chloroform/methanol according to Bligh and Dyer [31]. The lipid extracts (organic phases) were analyzed by TLC on LK6D chromatography plates (Whatman) in a solvent system composed of chloroform/methanol/acetic acid/water (75/45/12/3). Authentic phospholipid standards (Sigma) were run in parallel and detected with iodide vapors. Radioactivity in lipid spots was determined by using an automatic linear radiochromatography analyzer, Tracemaster 20 (Berthold), equipped with an 8-mm window and the integration software supplied by the manufacturer.

RESULTS

Kinetics of Surface Exposure of PtdSer in CD95-Treated Cells

Jurkat cells were treated with 0.5 μg/mL CD95 mAb, CH-11, and the exposure of PtdSer at the cell surface was monitored by labeling with annexin V–FITC and FACS analysis. The binding of annexin V–FITC was monitored from 0 to 180 min after CD95 mAb addition. Figure 1 shows that annexin V–FITC binding activity appeared on the cell surface after a lapse of time of about 75 min. Thereafter, the number of annexin V–FITC-positive cells

TABLE 1. Phosphatidylserine synthesis in Jurkat cells

Effectors	PtdSer synthesis (cpm ± SD)	PtdSer synthesis (%)
None	857 ± 25	100
CD3 (2 μg/mL)	385 ± 12	45
Thapsigargin (10^{-8}M)	171 ± 20	20
Quinine (50 μM)	1499 ± 122	175
Stearylamine (2.5 μM)	2571 ± 235	300
CPZ (20 μM)	1885 ± 87	220
TFP (20 μM)	2013 ± 131	235
W7 (50 μM)	1585 ± 123	185
NaN_3 (5 mM) + 2-DG (25 mM)	102 ± 45	12
Valinomycin (1 μM)	1371 ± 73	160
Gramicidin (1 μM)	179 ± 18	21
KCl (140 mM)	85.7 ± 32	10

Cells were incubated in the presence of [^{3}H]serine and effectors for 1 hr and the cellular phospholipids then extracted with chloroform–methanol and separated by TLC. The amount of [^{3}H]serine incorporated into PtdSer was monitored with a TLC radiochromatography scanner. Each value corresponds to the mean ± SD (N = 9) of three experiments, each done in triplicate. 2-DG, 2-deoxyglucose.

increased with time, reaching 80 ± 14% (N = 3) at 180 min. Control cells remained annexin V–FITC-negative during the duration of the experiment.

Effect of a Decreased PtdSer Synthesis on CD95-Induced PtdSer Exposure at the Cell Surface

Activation of Jurkat T cells with CD3 mAb results in a marked decrease of [^{3}H]serine incorporation into PtdSer in the absence of modification of [^{3}H]serine uptake by the cells [19]. This inhibition of PtdSer synthesis occurred as the result of emptying intracellular Ca^{2+} stores, because the serine–BEES is located in the endoplasmic reticulum and is a Ca^{2+}-dependent enzyme [20]. By-passing the early CD3-induced signals with the blocker of the reticulum Ca^{2+}-ATPase, thapsigargin also resulted in a strong reduction of PtdSer synthesis (Table 1). To see whether a reduction in PtdSer synthesis affects CD95-induced exposure of this phospholipid on the external leaflet of the plasma membrane, cells were pretreated for 1 hr with 2 μg/mL CD3 mAb or 10^{-8} M thapsigargin followed by the addition of 0.5 μg/mL CD95 mAb for an additional period of 3 hr. Cell reactivity with annexin V–FITC was measured at the end of this 4-hr period. Figure 2 shows that treatment of Jurkat cells with CD3 mAb for 4 hr did not induce annexin V–FITC reactivity at the cell surface. As expected, CD95 added to the cells for 3 hr resulted in a strong binding of annexin V–FITC to the majority of cells. Interestingly, pretreatment of cells with CD3 mAb markedly reduced the number of CD95-induced annexin V–FITC-positive cells. By contrast, the intensity of the annexin V–FITC fluorescence was not affected. Similar results were obtained with thapsigargin (Fig. 3). Monitoring the number of propidium iodide-positive cells showed that the treatment marginally affected cell permeability, since the number of propidium iodide-positive cells never exceeded 1% (Fig. 2).

Effect of an Increase in PtdSer Synthesis on CD95-Induced PtdSer Exposure at the Cell Surface

Previous studies have shown that PtdSer synthesis could be up-regulated by several drugs. For example, we and others have shown that the calmodulin antagonists CPZ, TFP, and W7 [16, 24, 32, 33], the K^+ channel blocker quinine [18, 23, 34] and the cationic amphiphilic compound stearylamine [25, 35–37] were strong inductors of PtdSer synthesis in different cell lines. To determine whether these drugs affect CD95-induced PtdSer exposure, Jurkat T cells were treated for 1 hr with these drugs, then for 2 hr with CD95 mAb. The results obtained showed that all drugs tested increased both PtdSer synthesis (Table 1) and CD95-induced PtdSer exposure at the cell surface (Fig. 4). The drugs alone at the concentrations indicated in the figure legend and during the 3-hr period of the experiment did not induce annexin V labeling (Fig. 4) or permeability to propidium iodide or trypan blue (not shown).

Role of ATP in PtdSer Synthesis and PtdSer Exposure at the Cell Surface

As previously demonstrated, PtdSer synthesis requires ATP, probably because ATP is necessary for the activity of Ca^{2+}-ATPase, which maintains a high Ca^{2+} concentration in the endoplasmic reticulum [15, 38]. We thus measured the influence of ATP deprivation on both PtdSer synthesis and annexin V labeling. For this purpose, Jurkat T cells were maintained for 30 min in a glucose-free medium in the presence of NaN_3 and 2-deoxyglucose, a treatment known to reduce the cellular ATP level by 90%. Then, the cells were treated with CD95 and the appearance of PtdSer at the cell surface was monitored every 15 min for 2 hr. The results depicted in Fig. 5 clearly show that in the absence of

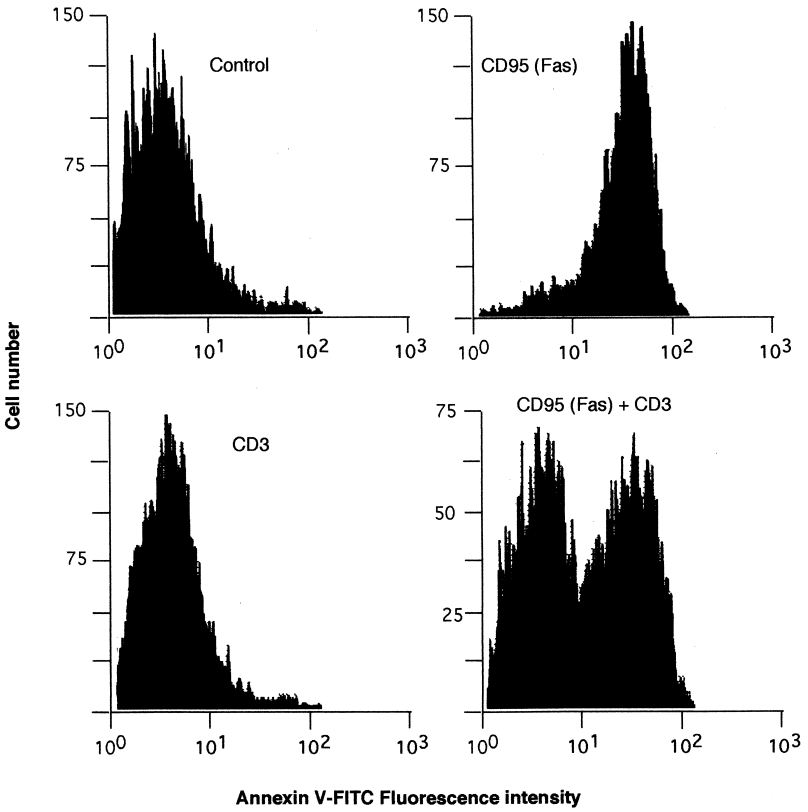

FIG. 2. Pretreatment with CD3 mAb reduces CD95-induced annexin V–FITC binding to Jurkat cells. Cells (10^6/mL) were treated for 1 hr with 2 μg CD3 mAb, then for 3 hr with 500 ng CD95 mAb. At the end of this incubation period, cells were labeled with annexin V–FITC and analyzed by cytofluorimetry. Controls as indicated in the figure included untreated cells, CD3-treated cells for 4 hr, and CD95-treated cells for 3 hr. The figure is representative of several experiments.

ATP the CD95-induced exposure of PtdSer was totally abrogated. Similarly, the PtdSer synthesis was inhibited by 90% (Table 1).

Role of Membrane Potential in PtdSer Synthesis and PtdSer Exposure at the Cell Surface

In a previous paper [26], we showed that PtdSer synthesis could be modulated by changing the plasma membrane potential. In this case, the strong inhibition of PtdSer synthesis observed was mainly due to a decreased transport of [^{3}H]serine at the plasma membrane level. To observe the influence of plasma membrane potential on CD95-induced PtdSer exposure, cells were treated with either valinomycin, which hyperpolarizes the cells, or with gramicidin, which causes a severe depolarization. In addition, replacement of NaCl with KCl in the medium, another means to depolarize the cells, was performed. As shown in Fig. 6, CD95-induced exposure of PtdSer was increased by valinomycin treatment, whereas it was markedly decreased by the depolarization induced by both gramicidin and NaCl replacement. As previously described (see Table 1), PtdSer synthesis was either increased by valinomycin or decreased by gramicidin or KCl treatments.

DISCUSSION

PtdSer, generally sequestered in the plasma membrane inner leaflet, appears in the outer leaflet during apoptosis and is recognized by macrophages as a signal to remove apoptotic cells [4, 39–41]. Annexin V–FITC specifically recognizes PtdSer–Ca^{2+} complexes [42] and is widely used to detect early apoptotic cells by cytofluorimetry [43, 44]. The mechanism(s) of PtdSer translocation to the external leaflet of the membrane during CD95-induced apoptosis is (are) not well understood, and its possible regulation has been poorly studied. It has been suggested that a decreased activity of the aminophospholipid translocase, the protein(s) responsible for the internal localization of PtdSer, participates in PtdSer externalization [45]. In addition, a phospholipid scramblase has been proposed as a candidate to regulate PtdSer movements to the cell surface [46]. During CD95-induced apoptosis, it seems clear that PtdSer exposure at the cell surface primarily involves the activation of a cysteine protease cascade (caspases) [7, 47, 48]. Caspase-8 and caspase-3 appear to be key enzymes, turning on the mechanism(s) leading to PtdSer exposure at the cell surface [49].

In the present work, we have examined the possibility that the level of serine–BEES activity (the enzyme respon-

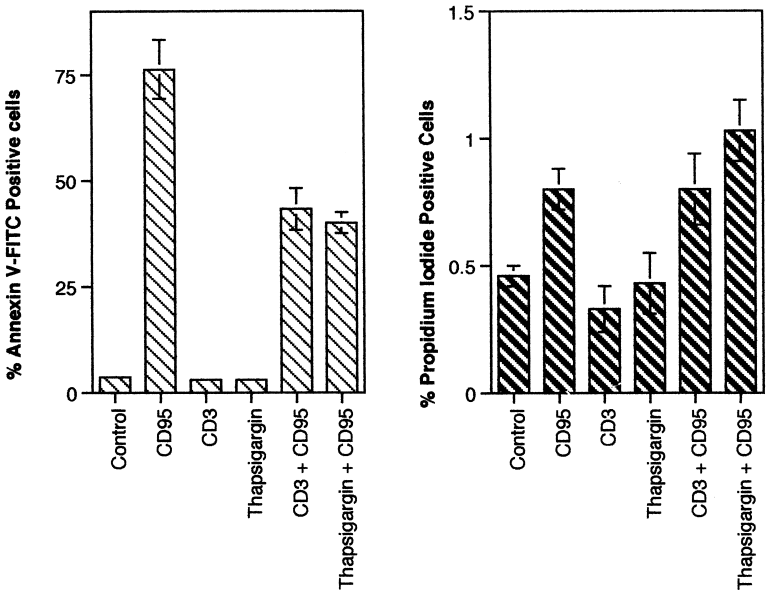

FIG. 3. CD3 mAb and thapsigargin reduce the number of CD95-induced annexin V–FITC-positive cells. Annexin V–FITC- and propidium iodide-positive cell numbers were measured by cytofluorimetry in controls and in cells treated for 2 hr with CD95 (500 ng/mL), CD3 (2 μg/mL), or thapsigargin (10^{-8} M), alone or in combination. CD3 or thapsigargin was applied for 1 hr followed by 2-hr treatment with CD95. Note that the number of propidium iodide-positive cells remained inferior to 1%. Each bar corresponds to the mean ± SD of two experiments done in triplicate.

sible for PtdSer synthesis) could regulate CD95-induced PtdSer exposure at the surface of Jurkat T cells. In our previous papers, we showed that PtdSer synthesis is down-regulated during T cell activation through the CD3/T cell receptor complex. The mechanism of this inhibition involves the well-known CD3-induced release of Ca^{2+} from the endoplasmic reticulum (ER). Indeed, the serine–BEES is a Ca^{2+}-dependent enzyme located in the ER, and emptying the intracellular Ca^{2+} stores (ER) results in a strong inhibition of PtdSer synthesis. A similar result can be obtained with the Ca^{2+}-ATPase inhibitor, thapsigargin, which by-passes the early activation signals generated through the CD3/TCR. We have found (Figs. 1 and 2) that a pretreatment with either CD3 mAb or thapsigargin

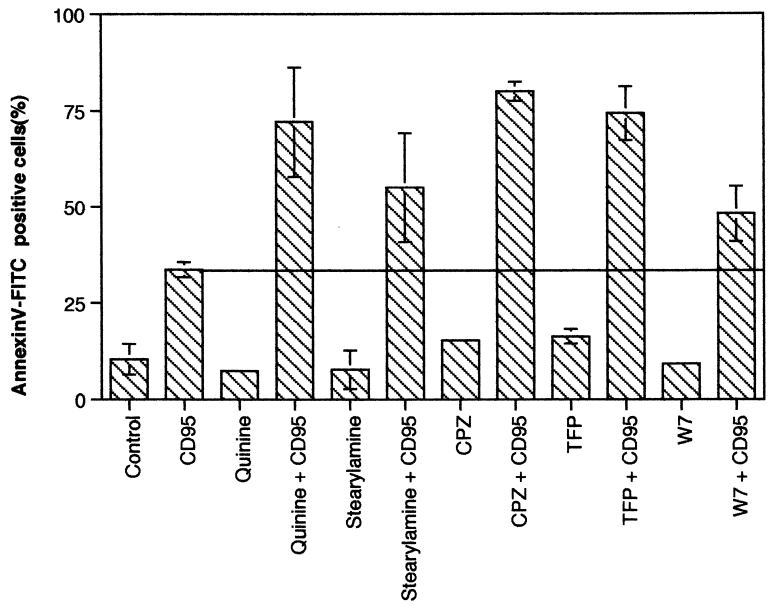

FIG. 4. Drugs increasing PtdSer synthesis also increase the percentage of CD95-induced annexin V–FITC-positive cells. Jurkat cells were treated for 1 hr with either 50 μM of the K^+ channel blocker quinine, 2.5 μM of the cationic amphiphilic drug stearylamine, or the calmodulin antagonists CPZ (20 μM), TFP (20 μM), or W7 (50 μM). CD95 (500 ng/mL) was then added for an additional period of 2 hr and annexin V–FITC-positive cell number measured by cytofluorimetry. The figure is representative of a typical experiment. Each bar represents the mean ± SD of triplicates.

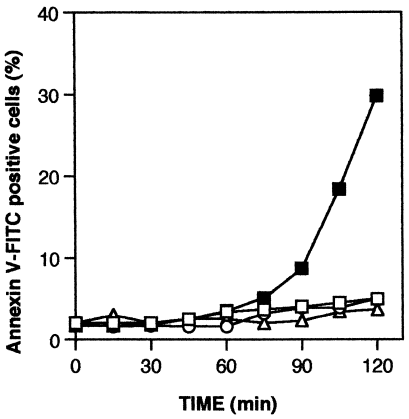

FIG. 5. Effect of ATP depletion on CD95-induced cell reactivity with annexin V–FITC. Cells were treated with 5 mM NaN_3 + 25 mM 2-deoxyglucose for 30 min in a glucose-free medium, after which 0.5 μg/mL CD95 mAb was added and the annexin V–FITC-positive cell number monitored every 15 min for 2 hr. (△), controls maintained in complete medium (with glucose); (■), cells treated with CD 95 in complete medium (with glucose); (□), cells treated with CD95 in the presence of NaN_3 and 2-deoxyglucose; and (○), control cells maintained in the presence of NaN_3 and 2-deoxyglucose. Each point represents the mean of three determinations.

markedly reduced the number of cells that react with annexin V–FITC during CD95-induced cell apoptosis. This result is consistent with a recent report [50] indicating that depletion of intracellular stores delays apoptosis in human neutrophils.

In a second set of experiments, we were interested to see whether an increased PtdSer synthesis could enhance CD95-induced PtdSer exposure. PtdSer synthesis in Jurkat cells could be largely increased by several drugs in the absence of significant modifications of [^{3}H]serine uptake by the cells. For example, some compounds generally used as K^+ channel blockers, such as quinine, increased PtdSer synthesis by 75%. The calmodulin antagonists chlorpromazine, trifluoperazine, and W7 and the cationic amphiphil stearylamine significantly enhanced PtdSer synthesis in Jurkat T cells (Table 1). The results obtained in the present work clearly indicate that increasing PtdSer synthesis also augments the annexin V–FITC reactivity of the plasma membrane in CD95-treated cells.

In an additional experiment, we stopped PtdSer synthesis by depleting the cell of ATP, according to a previous study by Czarny *et al.* [38]. Under these conditions, both PtdSer synthesis and CD95-induced PtdSer exposure at the cell surface were abrogated. This result confirms a previous report [51] that demonstrated that antimycin A and oligomycin, two mitochondrial inhibitors of ATP synthesis, abrogated PtdSer exposure at the surface of THP-1 and U937 cells undergoing apoptosis. Moreover, modifying PtdSer synthesis through modulation of [^{3}H]serine transport at the cell membrane either by hyperpolarization (valinomycin) or depolarization (gramicidin or KCl) resulted in changes in CD95-induced PtdSer exposure at the cell surface that reflected changes in PtdSer synthesis. A recent report [50] also indicated that membrane depolarization was associated with a delay of apoptosis in neutrophils. The present work confirms this fact and supports the idea that PtdSer synthesis is highly implicated in this phenomenon.

Even though the chemicals employed in this study have very different pharmacological properties, they all have the ability to stimulate PtdSer synthesis. Thus, it appears likely that the results obtained herein are due to the change in PtdSer synthesis rather than to a non-specific effect of the drugs. Moreover, since the drugs employed were unable by themselves to induce the annexin V–FITC reactivity of the cells, it appears unlikely that the drugs changed either the activity of the PtdSer translocase or the phospholipid scramblase activity responsible for PtdSer exposure. Con-

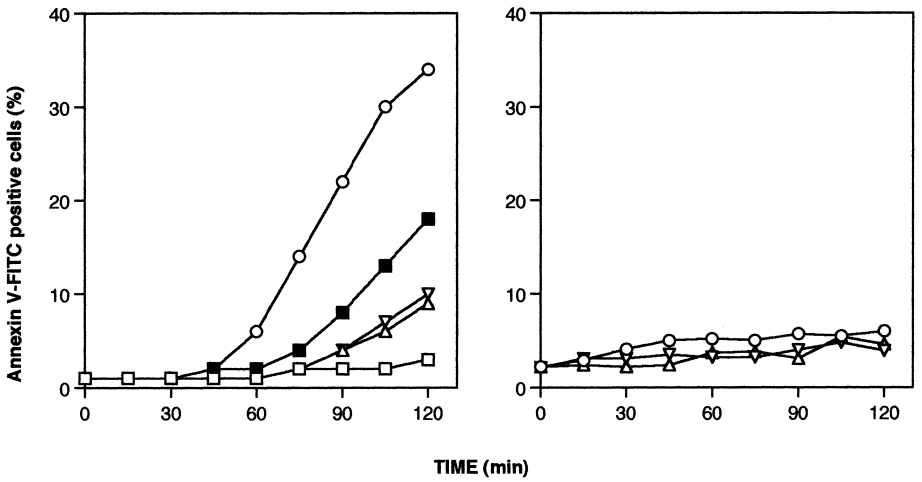

FIG. 6. Effects of changes in membrane potential on CD95-induced cell reactivity with annexin V–FITC. (Left panel) Annexin V–FITC reactivity of controls (□) and cells treated with CD95 alone (■) or with CD95 in the presence of 1 μM valinomycin (○), 1 μM gramicidin (△), or 140 mM KCl (▽). (Right panel) Controls corresponding to cells treated with valinomycin, gramicidin, or KCl alone. FACS analysis was performed every 15 min for a total of 2 hr. Other details as in Fig. 4.

cerning the possible activation of the phospholipid scramblase, the present study contradicts a previous work [52] that proposed a Ca^{2+}-mediated activation of the scramblase by high doses of the Ca^{2+} ionophore A23187. Indeed, in our hands, the use of CD3 mAb or of thapsigargin (two well-known procedures able to induce both a Ca^{2+} mobilization from intracellular Ca^{2+} stores and a strong Ca^{2+} influx) was not sufficient in itself to induce PtdSer exposure at the cell surface. In addition, it is important to note that CD95 induces PtdSer exposure at the cell surface, but is unable to induce Ca^{2+} mobilisation or Ca^{2+} entry into the cells and is also unable to modify CD3- or thapsigagin-induced Ca^{2+} movements [9]. Accordingly, it appears more likely that externalization of PtdSer possibly involves a reduction in the aminophospholipid translocase activity [45] rather than an activation of a Ca^{2+}-dependent phospholipid scramblase. Our studies on CD95-induced DNA fragmentation (not shown) indicated that the drugs used were unable to induce DNA ladders or decrease the binding of Hoechst 33342 within the duration of our experiments. In addition, the drugs were unable to modify CD95-induced DNA laddering and the CD95-induced decrease in Hoechst 33342 binding to DNA. Accordingly, neither inhibition nor stimulation of PtdSer synthesis interfered with the late events of the apoptotic process. This confirms a previous report [51] which indicated that PtdSer exposure at the cell surface and DNA fragmentation are two dissociated events of the apoptotic program.

All together, our results indicate that the amount of PtdSer exposed at the cell surface by triggering Fas with CD95 mAbs is at least in part regulated by the activity of the serine–BEES. Because the serine–BEES is the only mechanism used by the cell to synthesize PtdSer, it is tempting to speculate that the PtdSer content of the cells was modified by the different treatments used in this study. Nevertheless, attempts to measure changes in the total amount of PtdSer in the cells were unsuccessful because PtdSer is one of the major phospholipids in membranes, representing about 15% of all phospholipids. Changes in the activity of the serine–BEES as measured by the specific incorporation of [^{3}H]serine into PtdSer represents a small portion of total PtdSer. Accordingly, the measurement of [^{3}H]serine incorporation into PtdSer represents only the activity of the serine–BEES. Could the newly synthesized PtdSer be preferentially exposed at the cell surface? This question merits consideration, because we have previously shown [9] that CD95 induces both PtdSer synthesis and PtdSer exposure at the cell surface. It was found that about 40% of newly synthesized [3H] PtdSer was exposed at the cell surface as measured by derivatization of PtdSer with trinitrobenzene sulfonate (TNBS). Unfortunately, this technique was not usable in the present work because cells treated with the different drugs were too fragile. More precisely, in cells treated together with CD95, drugs that increased PtdSer synthesis, and TNBS, a derivatization of PtdSer by TNBS occurs on boths side of the membrane and possibly inside the cells, making the technique unusable in this peculiar situation.

Kinetics of PtdSer exposure as measured with annexin V–FITC, (see Fig. 6 for example) indicated that increasing the activity of the serine–BEES induced a 15-min reduction in the lapse of time necessary to induce PtdSer exposure under CD95 treatment. This indicates that cells that highly synthesize PtdSer are more sensitive to CD95 (in terms of PtdSer exposure) than those that poorly synthesize this phospholipid. This phenomenon might have important physiological consequences: for example, during a Fas–Fas ligand interaction, activated T cells would be less susceptible to elimination by macrophages than unactivated cells. In addition, during HIV infection, the well-known elimination of CD4-positive cells might use a similar mechanism, since it has been shown that CD4 mAbs and the gp120 protein of the HIV induce a marked increase in serine–BEES activity [53]. In addition, the fact that CD3-activated Jurkat cells present a decreased PtdSer synthesis and concomitantly become more resistant to CD95-induced PtdSer at the cell surface is in line with several previous observations [27–30] demonstrating that T cell activation down-regulated CD95-induced apoptosis.

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