# Fas-triggered phosphatidylserine exposure is modulated by intracellular ATP

Bettina Gleiss, Vladimir Gogvadze[1], Sten Orrenius, Bengt Fadeel*

*Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, SE-171 77 Stockholm, Sweden*

Received 12 April 2002; accepted 15 April 2002

First published online 25 April 2002

Edited by Barry Halliwell

**Abstract Recognition signals are displayed on the cell surface during apoptosis that enable macrophages to engulf and dispose of the dying cell. A common signal is the externalization of phosphatidylserine (PS). Studies in erythrocytes and platelets have suggested that PS exposure requires the concomitant activation of a phospholipid scramblase (PLS) and inhibition of an adenosine triphosphate (ATP)-dependent aminophospholipid translocase. However, the molecular mechanism underlying PS exposure during apoptosis remains poorly understood. In this study, we provide evidence that expression of PLS is neither necessary nor sufficient for PS exposure during Fas-triggered apoptosis. On the other hand, egress of PS is shown to correlate with a decline in intracellular ATP and inhibition of aminophospholipid translocase activity upon Fas stimulation. Moreover, suppression of intracellular ATP levels by the glucose antimetabolite, 2-deoxyglucose, alone or in combination with glucose-free medium, potentiates Fas-induced PS exposure in the PLS-expressing Jurkat cell line and enables PLS-defective Raji cells to externalize PS in response to Fas ligation. These studies suggest that intracellular ATP levels can modulate the externalization of PS during apoptosis, and implicate the ATP-dependent aminophospholipid translocase in this process.** 

*Key words:* Aminophospholipid translocase; Apoptosis; Adenosine triphosphate; Phosphatidylserine; Phospholipid scramblase

## 1. Introduction

Eukaryotic cells are characterized by an asymmetric distribution of their membrane phospholipids [1]. Hence, the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) are normally confined to the inner leaflet of the plasma membrane, whereas phosphatidylcholine and sphingomyelin are located predominantly in the outer leaflet. Phospholipid asymmetry is relatively stable and is believed to be maintained by an aminophospholipid translocase, which specifically transports PS and PE from the outer to the inner leaflet of the plasma membrane [2]. This adenosine triphosphate (ATP)-dependent and $Ca^{2+}$-inhibitable enzyme activity is sensitive to the sulfhydryl-reactive agent *N*-ethylmaleimide (NEM) and to vanadate, an inhibitor of P-type ATPases [2]. A candidate protein termed ATPase II with phospholipid transporting properties was recently identified, yet whether or not this protein is responsible for aminophospholipid translocation in the plasma membrane remains to be determined [3,4].

Externalization of PS has been shown to occur in activated platelets and in aging erythrocytes and serves to stimulate the coagulation cascade and mediate cell recognition by macrophages, respectively [2]. PS externalization is also a common event during apoptosis [5,6], and previous studies have demonstrated that PS exposure in cells undergoing apoptosis is caspase-dependent and requires extracellular $Ca^{2+}$ [7–9]. The rapid externalization of PS during platelet activation is thought to be mediated by a $Ca^{2+}$-dependent plasma membrane protein, phospholipid scramblase (PLS), which catalyzes the bidirectional movement of phospholipids between membrane leaflets [2]. Zhao et al. [10] have recently documented a close correlation between $Ca^{2+}$ ionophore-triggered PS exposure and the expression of PLS in a number of tumor cell lines. However, we have previously shown that expression of PLS in tumor cells does not correlate with the externalization of PS in response to Fas stimulation [11]. These observations, which have been corroborated in recent studies by other investigators [12], thus indicate that the mechanism of delayed PS exposure during apoptosis differs from the rapid, $Ca^{2+}$-stimulated PS exposure evidenced in activated platelets. In the present study, we sought to further characterize the mechanism of Fas-induced exposure of PS using two Fas-sensitive cell lines expressing different levels of PLS. Our data show that expression of PLS is neither necessary nor sufficient for PS exposure during apoptosis, and suggest that inhibition of the ATP-dependent aminophospholipid translocase plays a critical role in the process of PS externalization.

## 2. Materials and methods

### 2.1. Reagents and cell culture

Anti-Fas monoclonal antibodies (clone CH-11) were purchased from Medical and Biological Laboratories Co., Ltd. (Nagoya, Japan). zVAD-fmk (benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone) and

*Corresponding author. Fax: (46)-8-32 90 41.
*E-mail address:* bengt.fadeel@imm.ki.se (B. Fadeel).

[1] Permanent address: Institute of Theoretical and Experimental Biophysics, Pushchino 142290, Russia.

*Abbreviations:* ATP, adenosine triphosphate; DEVD-AMC, Asp-Glu-Val-Asp-7-amino-4-methylcoumarin; 2-DG, 2-deoxyglucose; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN, interferon; NBD, 1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl) amino]caproyl]-*sn*-glycero-3; NEM, *N*-ethylmaleimide; PLS, phospholipid scramblase; PS, phosphatidylserine; zVAD-fmk, benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone

0014-5793/02/$22.00 
PII: S0014-5793(02)02743-6

DEVD-AMC (Asp-Glu-Val-Asp-7-amino-4-methylcoumarin) were obtained from Enzyme Systems Products (Dublin, CA, USA) and Peptide Institute, Inc. (Osaka, Japan), respectively. NEM, 2-deoxyglucose (2-DG) and oligomycin were purchased from Sigma (St. Louis, MO, USA). Recombinant human interferon (IFN)-α was kindly provided by Dr. Stefan Einhorn, Karolinska Hospital, Stockholm, Sweden. The human leukemic T cell line Jurkat and the EBV-transformed human B cell line Raji were from the European Collection of Cell Cultures (Salisbury, UK). Cells were cultured in RPMI-1640 medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (Life Technologies).

### 2.2. PS exposure

Exposure of PS was determined by the binding of FLUOS-coupled annexin V (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's protocol. Briefly, treated cells ($0.5\times10^6$) were washed and resuspended in binding buffer containing annexin V-FLUOS and propidium iodide for 10 min prior to analysis. Ten thousand events were collected on a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with a 488 nm argon laser, and analyzed using the CellQuest software (Becton Dickinson). Low-fluorescence debris and necrotic cells, defined as cells that had lost their membrane integrity and thus were permeable to propidium iodide, were gated out prior to analysis.

### 2.3. Caspase activity

The measurement of DEVD-AMC cleavage was performed in a fluorometric assay as previously described [11]. Briefly, cell lysates ($1.0\times10^6$) and substrates were combined in a reaction buffer (100 mM HEPES, 10% sucrose, 5 mM dithiothreitol, $10^{-6}$% NP-40 and 0.1% CHAPS; pH 7.25) and added to a microtiter plate. AMC liberation was monitored in a Fluoroscan II plate reader (Labsystems, Stockholm, Sweden) using 355 nm excitation and 460 nm emission wavelengths. Fluorescence was measured every 70 s during a 30 min period and fluorescence units were converted to pmol of AMC using a standard curve generated with free AMC. Data from duplicate samples were then analyzed by linear regression.

### 2.4. Aminophospholipid translocase activity

Aminophospholipid translocase activity was measured as the uptake of the fluorescence-labeled phospholipid 1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl) amino]caproyl]-*sn*-glycero-3-phosphoserine (NBD-PS) (Avanti Polar-Lipids, Inc., Alabaster, AL, USA) as described by Bratton et al. [13]. Briefly, treated cells ($5.0\times10^5$) were washed once and resuspended in 50 μl HEPES-buffered saline (HBS) (137 mM NaCl, 2.7 mM KCl, 2 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES, pH 7.4, with 1 mM $CaCl_2$). The cells were incubated with 1 μl of NBD-PS (50 μg/ml) and 1 μl of propidium iodide (250 μg/ml) for 10 min at room temperature, followed by back extraction with 50 μl of 1% bovine serum albumin (BSA) in HBS for 5 min. Samples were then transferred to ice, 900 μl of ice-cold HBS was added and analysis was performed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA).

### 2.5. ATP determination

ATP concentrations were determined in a luminometric assay using the ATP dependency of the light-emitting luciferase-catalyzed oxidation of luciferin (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's protocol. Briefly, cells ($5.0\times10^4$) were

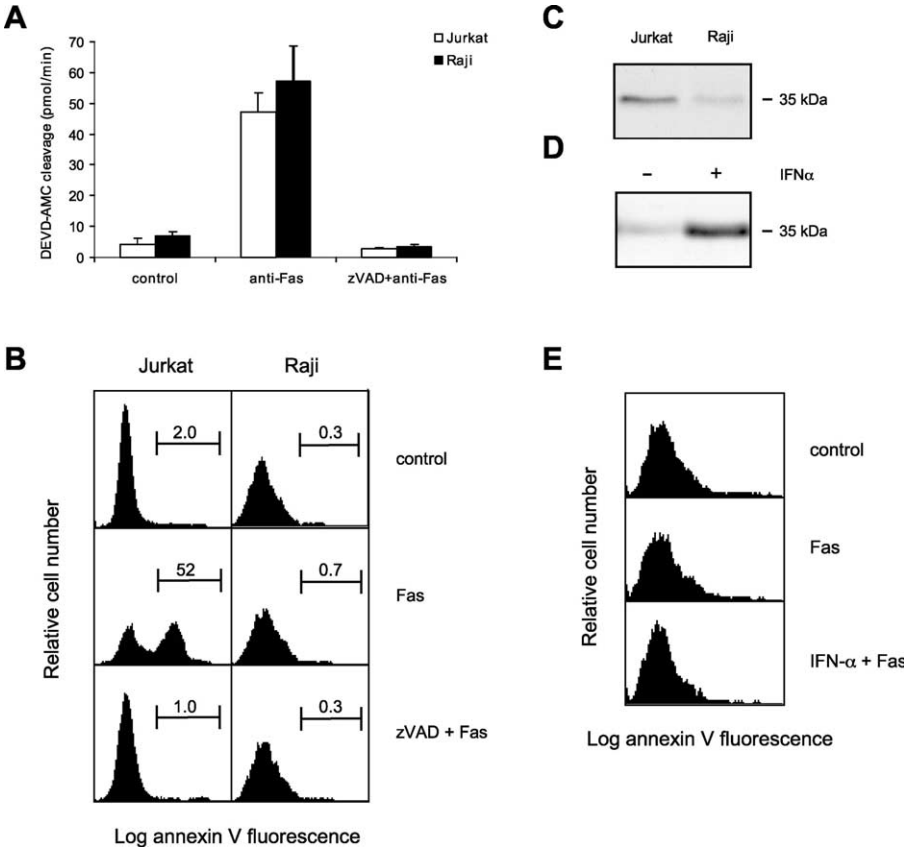


Fig. 1. Fas-triggered PS exposure in Jurkat, but not in Raji cells: induction of PLS fails to facilitate PS exposure in Raji cells. A: Caspase 3-like activity, expressed as pmol of AMC released per min, in cells treated with anti-Fas antibodies (250 ng/ml) for 4 h in the presence or absence of zVAD-fmk (10 μM). Data are expressed as mean ± S.D. ($n=3$). B: PS exposure, as detected by flow cytometric analysis of annexin V binding, in Jurkat and Raji cells treated as above. The percentage of annexin V-positive cells in each sample is indicated. Data are representative of at least five separate experiments. C: Western blot analysis of PLS expression (35 kDa) in equal numbers of untreated Jurkat and Raji cells. D: Upregulation of endogenous PLS in Raji cells upon stimulation with IFN-α (1000 IU/ml) for 6 h. Cell lysates derived from an equal number of cells were loaded in each lane. E: Annexin V staining of Raji cells incubated in the presence or absence of IFN-α as above and then stimulated with anti-Fas antibodies (250 ng/ml) for 6 h. Data shown are representative of at least four separate experiments.

added to 1 ml of boiling lysing buffer (100 mM Tris, 4 mM EDTA, pH 7.75), samples were incubated for another 2 min at 100°C and 50 μl were taken out to a 96-well plate. Prior to measurement, 50 μl of luciferase were added to each well and the plate was analyzed in a luminometer (Anthos, Labtec Instruments, Austria).

### 2.6. Reverse transcription (RT)-PCR

Total RNA was isolated from $10^7$ cells, using the RNeasy Mini kit (Qiagen) according to the manufacturer's protocol. 5 μg of total RNA were subjected to RT coupled with a PCR reaction, performed in a DNA Thermal Cycler 480 (Perkin Elmer), using the SuperScript II RNase H$^-$ RT-PCR system (Life Technologies). Primers used for ATPase II were: P1 (sense primer) 5′-CTG GTA CTC TGA CAT GCA ATG-3′ and P2 (antisense primer) 5′-GAG ATT TTC CAG CAA TGA TGA ATC-3′ (Life Technologies) [4]. The cycling conditions were 94°C for 3 min and *n* (24–27) cycles consisting of 94°C for 15 s, 55°C for 30 s and 72°C for 30 s. After the last cycle, incubation at 72°C was prolonged for 7 min. PCR reactions for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were performed separately using the following primers: 5′-ACC ACA GTC CAT GCC ATC AC-3′ and 5′-TCC ACC ACC CTG TTG CTG TA-3′ (Life Technologies). The cycling conditions were 94°C for 3 min and 19 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 45 s. Again, incubation at 72°C was prolonged for 7 min after the last cycle. PCR products (170 and 452 bp, respectively) were resolved on a 2.5% agarose gel and visualized with ethidium bromide staining. The intensity of the bands was quantified using a Gel-Doc densitometer (Bio-Rad) connected to the Molecular Analyst software.

### 2.7. Western blot

For protein detection, samples were solubilized for 5 min at 100°C in Laemmli buffer. Proteins were then separated at 130 V on 12% SDS–polyacrylamide gels and electrophoretically transferred to nitrocellulose filters for 2 h at 100 V. Membranes were blocked overnight in 1% BSA and 5% non-fat dried milk and then incubated for 1 h with monoclonal antibodies against PLS (1:1000; provided by Dr. Peter Sims, The Scripps Research Institute, La Jolla, CA, USA) or polyclonal antibodies directed against GAPDH (1:3000; Trevigen, Gaithersburg, MD, USA). After washing, the membranes were incubated with a peroxidase-conjugated secondary antibody (1:10 000; Pierce, Rockford, IL, USA) and bound antibody was visualized by enhanced chemiluminescence (Amersham Corp., Buckinghamshire, UK) according to the manufacturer's instructions.

## 3. Results

### 3.1. Fas-triggered PS exposure in Jurkat, but not in Raji cells: induction of PLS fails to facilitate PS exposure

Jurkat and Raji cells both readily undergo apoptosis with attendant activation of caspase 3-like enzymes in response to Fas ligation, yet only Jurkat cells externalize PS (Fig. 1A,B). As expected, the broad range caspase inhibitor, zVAD-fmk (10 μM), which blocks hydrolysis of the caspase substrate DEVD-AMC (Fig. 1A), prevented PS exposure in Jurkat cells (Fig. 1B). PLS is known to regulate PS externalization in response to $Ca^{2+}$ stimulation [2]. Interestingly, Raji cells express very low levels of PLS while this protein is readily detected in Jurkat cells (Fig. 1C). However, in line with previous studies [11], induction of PLS by IFN-α (1000 IU/ml) stimulation of Raji cells did not confer the ability to expose PS upon Fas ligation (Fig. 1D,E). Due to a lack of suitable reagents, we were unable to determine the level of expression of the candidate aminophospholipid translocase, ATPase II, in Jurkat and Raji cells. RT-PCR analyses, on the other hand, revealed the presence of ATPase II mRNA in both cell lines, with an almost two-fold higher level of mRNA in Raji cells (Fig. 2A,B). Moreover, incubation of Jurkat and Raji cells with the thiol-reactive agent NEM (5 mM), a known inhibitor of the aminophospholipid translocase, resulted in a time-dependent induction of PS exposure in both cell lines (Fig. 2C). Taken together, these observations suggest that expression of

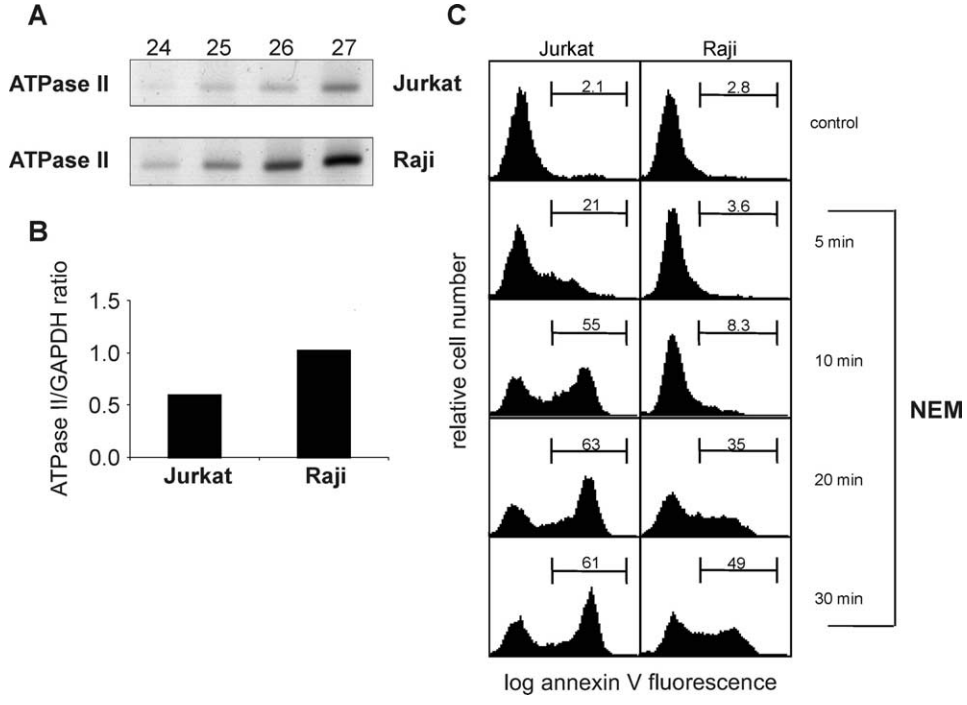


Fig. 2. Expression of the candidate aminophospholipid translocase, ATPase II, in Jurkat and Raji cells, and induction of PS exposure in both cell lines following NEM treatment. A: Total RNA from Jurkat and Raji cells was submitted to RT and PCR for 24–27 cycles using ATPase II-specific primers and PCR products were resolved on an agarose gel. B: Ratio between ATPase II after 27 PCR cycles and the internal control GAPDH. Data are representative of three independent experiments. C: Annexin V staining of Jurkat and Raji cells upon incubation with NEM (5 mM), an inhibitor of aminophospholipid translocation. Data are representative of four separate experiments.

PLS is not sufficient for apoptotic PS exposure, and emphasize the importance of inhibition of the aminophospholipid translocase, and/or of other thiol-dependent processes, for cell surface exposure of PS.

### 3.2. *Fas-mediated decline in ATP and concomitant suppression of the aminophospholipid translocase correlates with the ability to externalize PS*

To determine aminophospholipid translocase activity in Jurkat and Raji cells, we measured uptake of exogenous, fluorescently labeled NBD-PS. As seen in Fig. 3A, NEM (5 mM) blocked translocation of NBD-PS in both cell lines. In Jurkat cells, a time-dependent decrease in aminophospholipid translocase activity was observed upon Fas-triggering (Fig. 3B). In contrast, in Raji cells stimulated with anti-Fas antibodies, translocase activity was inhibited to a much lesser degree. Importantly, Fas-mediated suppression of translocase activity in Jurkat cells was completely abrogated by zVAD-fmk (Fig. 3B). Previous studies in erythrocytes have demonstrated that the inward movement of PS across the plasma membrane is inhibited by decreased levels of ATP [2]. These observations prompted us to study the level of intracellular ATP in Jurkat and Raji cells in response to Fas ligation. As shown in Fig. 3C, the ATP level in Jurkat cells decreased prominently dur-

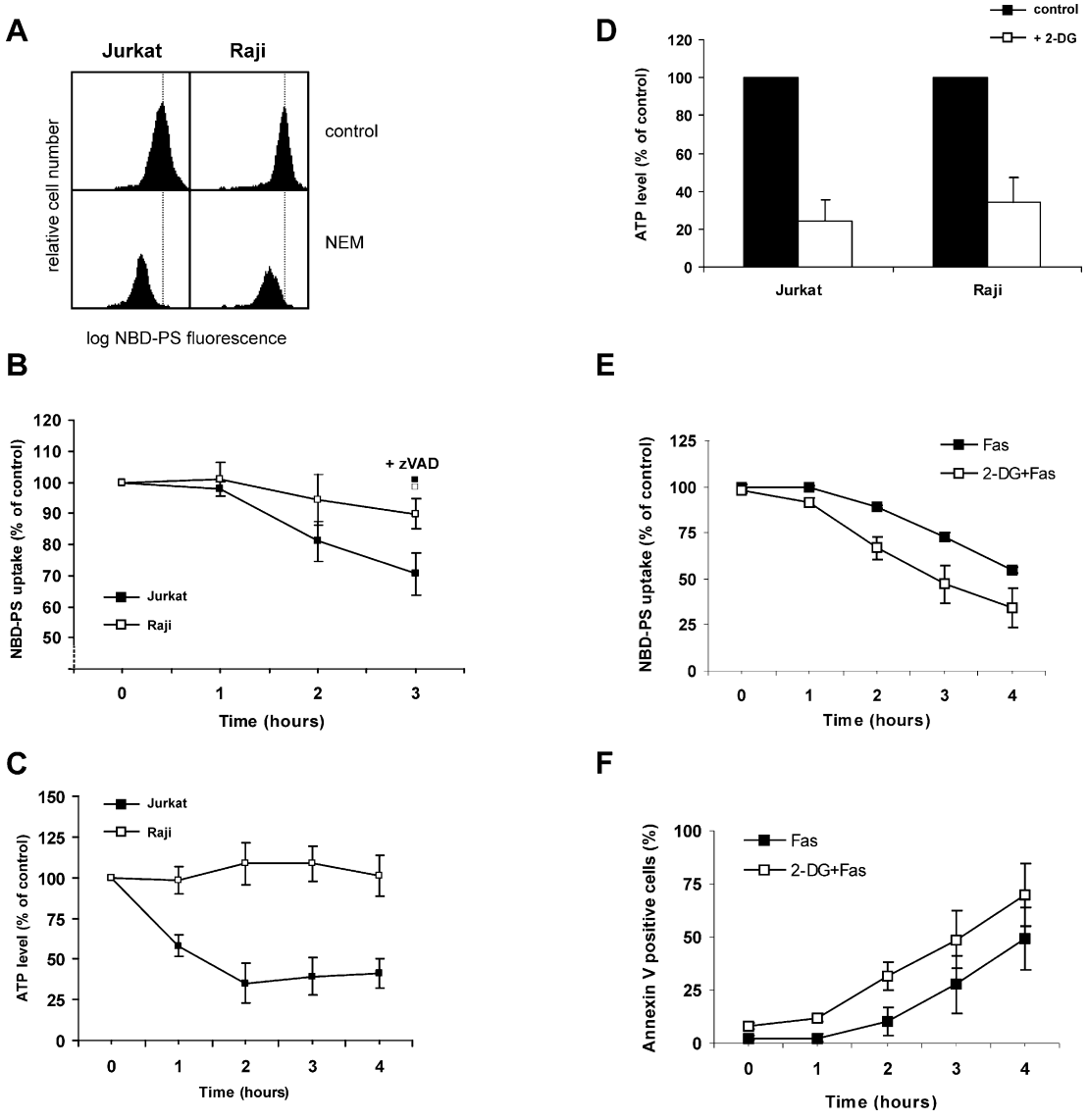


Fig. 3. PS exposure correlates with the Fas-triggered inhibition of aminophospholipid translocation and concomitant decline in intracellular ATP. Aminophospholipid translocase activity, as measured by the uptake of exogenous NBD-PS, was determined in Jurkat and Raji cells treated with (A) NEM for 5 min at 0.5 and 0.1 mM, respectively, or (B) anti-Fas antibodies (250 ng/ml) for the indicated times. Samples incubated for 3 h in the presence of zVAD-fmk (10 μM) plus anti-Fas antibodies are also shown. Values are given as the number of cells with NBD-PS uptake (% of control). C: Jurkat and Raji cells were incubated with anti-Fas antibodies (250 ng/ml) for the indicated times and intracellular ATP levels were analyzed using the luciferase-based luminometric assay. ATP concentrations are expressed as the percentage of untreated controls. D: Intracellular ATP concentrations were determined in cells incubated in the presence or absence of 2-DG (5 mM) for 24 h. Jurkat cells preincubated in the presence or absence of 2-DG for 24 h and incubated with anti-Fas antibodies (250 ng/ml) for the indicated times, were assessed for aminophospholipid translocation (E) and PS externalization (F) by determination of NBD-PS uptake and annexin V staining, respectively. Data in B–F are shown as mean ± S.D. ($n = 3$–4).

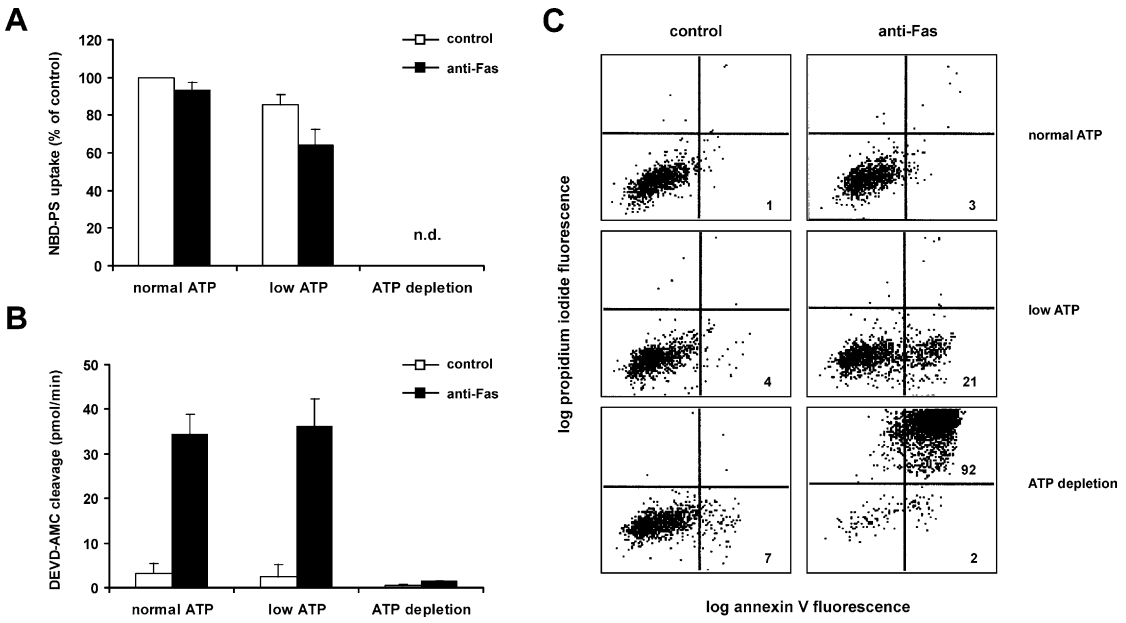


Fig. 4. Fas ligation triggers PS exposure in PLS-defective Raji cells under conditions of low intracellular ATP. Raji cells were preincubated either in regular medium (*normal ATP*) for 24 h, in glucose-free medium plus 2-DG (5 mM) for 24 h (*low ATP*), or in glucose-free medium plus oligomycin (10 μM) for 45 min (*ATP depletion*), and then incubated in the absence or presence of anti-Fas antibodies (250 ng/ml) for a further 12 h (*normal ATP*, *low ATP*) or 6 h (*ATP depletion*). Cells were subsequently analyzed for (A) aminophospholipid translocase activity, (B) activation of caspase-3-like enzymes, and (C) PS exposure, as described in Section 2. For clarity, necrotic cells were gated out in all samples prior to analysis of annexin V staining except in the case of Fas-triggered cells incubated under ATP-depleting conditions, for which only cell debris were excluded. Data shown in A and B are expressed as mean ± S.D. ($n = 3$). Data in C are representative of at least three independent experiments. n.d., not detectable.

ing the time of incubation with anti-Fas antibodies. However, ATP levels in Raji cells were maintained approximately at control values up to 4 h after apoptosis induction. To further investigate the relationship between ATP levels and PS exposure, both cell lines were incubated in medium containing 2-DG, a glucose analogue which competitively inhibits cellular uptake and utilization of glucose. Incubation with 2-DG for 24 h yielded a significant decrease in the level of ATP both in Jurkat and Raji cells (Fig. 3D). Moreover, preincubation of Jurkat cells with 2-DG, followed by Fas stimulation, potentiated the inhibition of aminophospholipid translocase activity as well as the degree of PS exposure (Fig. 3E,F). These events were accompanied by an increase in caspase-3-like activity, as determined by hydrolysis of the peptide substrate DEVD-AMC (data not shown). The present data thus demonstrate that Fas-triggered PS exposure can be modulated in Jurkat cells through suppression of intracellular ATP with concomitant inhibition of aminophospholipid translocase activity.

### 3.3. *Fas triggering of PLS-defective Raji cells under conditions of low intracellular ATP yields apoptotic PS exposure*

To investigate whether suppression of intracellular ATP levels would promote Fas-mediated PS exposure also in Raji cells, these cells were preincubated in glucose-free medium in combination with 2-DG for 24 h prior to stimulation with anti-Fas antibodies. These conditions dramatically enhanced the Fas-induced decrease in intracellular ATP ($< 10\%$ of ATP compared to untreated cells maintained in regular medium), and potentiated the Fas-induced inhibition of aminophospholipid translocase activity seen in these cells under normal incubation conditions (Fig. 4A). Remarkably, a fraction of Raji cells now exposed PS upon Fas ligation (Fig. 4C). This cell population was clearly apoptotic since cells were positive for annexin V yet remained negative for propidium iodide uptake; moreover, these cells maintained Fas-induced activation of caspase-3-like enzymes (Fig. 4B,C). By contrast, preincubation of Raji cells in glucose-free medium containing oligomycin, an inhibitor of the mitochondrial $F_0F_1$-ATPase, to deplete cells of ATP, resulted in the induction of necrosis and absence of caspase activation upon Fas stimulation (Fig. 4B,C). The latter data are thus consistent with previous studies in which ATP depletion was shown to redirect the mode of cell death from apoptosis to necrosis [14]. In sum, PLS-defective Raji cells, which normally fail to expose PS in response to Fas triggering, can do so when stimulated under conditions of low ATP.

## 4. Discussion

Previous studies have suggested that the concomitant inhibition of aminophospholipid translocation and activation of non-specific transbilayer movement of phospholipids (scrambling) coordinates the externalization of PS in cells undergoing apoptosis [15]. Furthermore, Bratton et al. [13] have proposed that loss of translocase activity is necessary, but not sufficient, for the appearance of PS on the surface of apoptotic cells and suggested that the activation of a scramblase (PLS) is required for PS exposure to occur. In contrast, we show herein that IFN-α-induced expression of PLS in Raji cells is insufficient for apoptotic PS exposure and provide evidence that Fas-triggered PS exposure is governed by the level of intracellular ATP in Jurkat and Raji cells, irrespective of PLS expression. Importantly, NEM, a thiol-reactive agent commonly used to inhibit aminophospholipid translocation [16], induced externalization of PS in both cell lines, in the absence of other signs of apoptosis. Moreover, suppression of

intracellular ATP levels potentiated translocase inhibition and PS exposure in Jurkat cells, and yielded Fas-induced PS exposure in the otherwise PS exposure-incompetent Raji cells. While we cannot exclude an effect on other ATP-dependent cellular processes, the current data nevertheless suggest that inhibition of the ATP-dependent aminophospholipid translocase, perhaps related to the inhibition of ATPase II, is sufficient to promote PS exposure during apoptosis.

As mentioned above, PLS activation is known to be involved in $Ca^{2+}$-stimulated PS exposure in activated platelets [2]. The current data obtained in Jurkat and Raji cells thus indicate that the mechanism of apoptotic PS exposure differs from the one evidenced in activated platelets insofar as expression of PLS appears to be dispensible in the former case. In further support of this view, Li and Tait [17] previously reported that anti-CD9 antibodies inhibit $Ca^{2+}$-stimulated PS exposure in Jurkat cells while PS exposure triggered by various apoptotic stimuli was unaffected. Interestingly, during the preparation of this manuscript, Silverman et al. [18] reported that IFN-α-inducible PLS affects the growth of human ovarian carcinoma cells in nude mice. While the mechanism by which PLS exerts its putative tumor suppressive function remains unresolved, these findings nevertheless support the notion that PLS may have alternative role(s), unrelated to PS externalization, in nucleated cells. Further elucidation of the different pathways that mediate PS externalization is expected to have important implications for our understanding of the recognition and clearance of dying cells, and may provide new insights into the pathogenesis and treatment of diseases in which cell clearance is perturbed.

*Acknowledgements:* We thank Drs. Peter Sims, The Scripps Research Institute, and Stefan Einhorn, Karolinska Hospital, for provision of PLS antibodies and recombinant IFN-α, respectively. Dr. John Robertson is acknowledged for critical reading of earlier versions of the manuscript. This work was supported by grants from the Swedish Medical Research Council, the Swedish Medical Society and Karolinska Institutet. V.G. was supported by a grant from the Royal Swedish Academy of Sciences.

## References

[1] Rothman, J.E. and Lenard, J. (1977) Science 195, 743–753.
[2] Bevers, E.M., Comfurius, P., Dekkers, D.W. and Zwaal, R.F. (1999) Biochim. Biophys. Acta 1439, 317–330.
[3] Tang, X., Halleck, M.S., Schlegel, R.A. and Williamson, P. (1996) Science 272, 1495–1497.
[4] Mouro, I., Halleck, M.S., Schlegel, R.A., Mattei, M.G., Williamson, P., Zachowski, A., Devaux, P., Cartron, J.-P. and Colin, Y. (1999) Biochem. Biophys. Res. Commun. 257, 333–339.
[5] Fadok, V.A., Voelker, D.R., Campbell, P.A., Cohen, J.J., Bratton, D.L. and Henson, P.M. (1992) J. Immunol. 148, 2207–2216.
[6] Martin, S.J., Reutelingsperger, C.P., McGahon, A.J., Rader, J.A., van Schie, R.C., LaFace, D.M. and Green, D.R. (1995) J. Exp. Med. 182, 1545–1556.
[7] Martin, S.J., Finucane, D.M., Amarante-Mendes, G.P., O'Brian, G.A. and Green, D.R. (1996) J. Biol. Chem. 271, 28753–28756.
[8] Vanags, D.M., Pörn-Ares, M.I., Coppola, S., Burgess, D.H. and Orrenius, S. (1996) J. Biol. Chem. 271, 31075–31085.
[9] Hampton, M.B., Vanags, D.M., Pörn-Ares, M.I. and Orrenius, S. (1996) FEBS Lett. 399, 277–282.
[10] Zhao, J., Zhou, Q., Wiedmer, T. and Sims, P.J. (1998) J. Biol. Chem. 273, 6603–6606.
[11] Fadeel, B., Gleiss, B., Högstrand, K., Chandra, J., Wiedmer, T., Sims, P.J., Henter, J.-I., Orrenius, S. and Samali, A. (1999) Biochem. Biophys. Res. Commun. 266, 504–511.
[12] Zhou, Q., Zhao, J., Al-Zoghaibi, F., Zhou, A., Wiedmer, T., Silverman, R.H. and Sims, P.J. (2000) Blood 95, 2593–2599.
[13] Bratton, D.L., Fadok, V.A., Richter, D.A., Kailey, J.M., Guthrie, L.A. and Henson, P. (1997) J. Biol. Chem. 272, 26159–26165.
[14] Leist, M., Single, B., Castoldi, A.F., Kuhnle, S. and Nicotera, P. (1997) J. Exp. Med. 185, 1481–1486.
[15] Verhoven, B., Schlegel, R.A. and Williamson, P. (1995) J. Exp. Med. 182, 1597–1601.
[16] van Engeland, M., Kuijpers, H.J.H., Ramaekers, F.C.S., Reutelingsperger, C.P.M. and Schutte, B. (1997) Exp. Cell Res. 235, 421–430.
[17] Li, W. and Tait, J.F. (1998) Arch. Biochem. Biophys. 351, 89–95.
[18] Silverman, R.H., Halloum, A., Zhou, A., Dong, B., Al-Zoghaibi, F., Kushner, D., Zhou, Q., Zhao, J., Wiedmer, T. and Sims, P.J. (2002) Cancer Res. 62, 397–402.