Monoclonal Antibodies Directed against the E2 Protein (MIC2 Gene Product) Induce Exposure of Phosphatidylserine at the Thymocyte Cell Surface†

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ABSTRACT: Monoclonal antibodies (mAbs) directed against E2, a 32-kDa transmembrane protein encoded by the MIC2 gene located in the pseudoautosomal region, induce a transbilayer movement of phosphatidylserine and, to a lesser extent, phosphatidylethanolamine in human thymocytes and a Jurkat T lymphocytes. The translocation of phosphatidylserine has been evidenced by using either derivatization of anionic phospholipids with trinitrobenzenesulfonate (TNBS) or cytofluorimetry after labeling of cells with anti-phosphatidylserine antibodies. The perturbation of membrane phospholipids induced by anti-E2 mAbs was further evidenced by labeling the cells with merocyanine 540. The specificity of anti-E2-induced perturbations of membrane asymmetry was tested by using a number of mAbs able to activate T cells, including CD3 and CD2. The results strongly suggest that anti-E2-induced changes in PtdSer are related to cell aggregation since the same mAbs specifically induce the aggregation of both thymocytes and Jurkat cells and since the E2 molecule has been previously implicated in the adhesive properties of human T cells with erythrocytes.

The asymmetric distribution of phosphatidylserine (PtdSer)[1] in the cell membrane is a well-known phenomenon. PtdSer resides exclusively in the cell inner leaflet (Verklij et al., 1973; Gordesky et al., 1975). The asymmetric distribution of PtdSer is maintained by an aminophospholipid translocase and is an ATP-dependent process (Connor et al., 1992; Devaux, 1991). The asymmetric distribution of PtdSer is lost during platelet aggregation induced by either collagen (Thiagarajan & Tait, 1991), thrombin (Rote et al., 1993), or collagen plus thrombin (Bevers et al., 1989).

The E2 antigen, a 32-kDa glycoprotein, is encoded by the MIC2 gene located in the pseudoautosomal region and shares no homology with any known family of proteins (Gelin et al., 1989a). It is worth noting that the E2 molecule displays isomorphism and that four distinct epitopes can be defined on E2. Two epitopes have a broad distribution on T cells and are involved in adhesion functions of E2 (Gelin et al., 1989a; Aubrit et al., 1989), whereas the two epitopes restricted within the T cell series to functionally specialized subsets apparently are not involved in the adhesion function. Recently monoclonal antibodies directed against the E2 protein have been shown to produce homotypic aggregation of human thymocytes and Jurkat T lymphocytes (G. Bernard, D. Zoccola, M. Deckert, J.-P. Breittmayer, C. Aussel, V. Lussiez, and A. Bernard, manuscript in preparation). This prompted us to study whether monoclonal antibodies directed against E2 modify PtdSer distribution at the surface of thymocytes. We report here the action of three monoclonal antibodies, L129, O662, and 12E7, recognizing three different epitopes on the E2 molecule (Gelin et al., 1991). Among them, only L129 and O662 induced a loss of PtdSer asymmetry in thymocytes and Jurkat cells. T cell activation induced by either CD3 or CD2 mAbs does not modify the distribution of PtdSer, indicating that the Ca^{2+} mobilization induced by these mAbs is not involved in this process.

MATERIALS AND METHODS

Cells. Jurkat D: The human T cell line Jurkat was kindly supplied by Dr. A. M. Schmitt-Verhulst (Centre d'Immunologie, Marseille-Luminy, France). Cells were cloned by limiting dilution. Clone D was selected on the basis of its IL-2 production when activated with phytohemagglutinin (PHA) and the phorbol ester, TPA. Cells were cultured in RPMI 1640 (Seromed, Lille, France) supplemented with 5% fetal calf serum, 50 units/mL penicillin, 50 mg/mL streptomycin, 2 mM L-glutamine, 1 mM pyruvate, and 0.1 mM β-mercaptoethanol.

Human T lymphocyte purification: T cells were extensively purified from peripheral blood as previously described (Huet et al., 1986). Briefly, after a 1-h adherence of PBL to plastic Petri dishes, nonadherent cells were removed and incubated with L-leucine methyl ester (5 mM). Residual monocytes and B cells were next eliminated by two cycles of treatment with a nontoxic rabbit complement and a mixture of CD14 and CD19 (kindly supplied by J. Wijdenes, Besancon, France), CD57 (HNK1), and anti-HLADR (G157) mAb. Immunomagnetic negative selection was then performed using Dynabeads (Dynal Inc., Fort Lee, NJ) recovered with goat anti-mouse IgG. Cells attached to the magnetic beads were removed using a rare earth magnet. The purity of the T cells was >95% as determined by flow cytometry analysis (FACScan, Becton-Dickinson, Mountain View, CA). Furthermore, the cells did not respond to PHAp (Wellcome, London, UK) at optimal concentration (1 mg/mL).

Human thymocytes were from thymic tissue obtained from children (<3 years) undergoing cardiac surgery. Mononuclear cell suspensions were obtained by teasing thymic samples and washed three times before use.

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[1] Abbreviations: PtdSer, phosphatidylserine; PtdEtn, phosphatidylethanolamine; TNBS, trinitrobenzenesulfonate; MC540, merocyanine 540; mAbs, monoclonal antibodies.

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Monoclonal Antibodies. The O662 (IgG3) and L129 (IgM) mAbs were prepared as previously described (Aubrit et al., 1989). The 12E7 (IgG2a) mAb was kindly provided by Dr. R. Levy (Levy et al., 1979). The CD3 mAb, X35 (IgG2a), was kindly provided by Dr. D. Bourrel (CRTS, Rennes, France). The CD2 mAbs, O275 (IgG2a), X11 (IgG1), GT2 (IgG1), and D66 (IgM), were produced in our laboratory as previously described (Valentin et al., 1989).

Metabolic Labeling of Phospholipids and Derivatization. Cells were labeled with either 4 μCi of L-[3-^{3}H]serine (0.37–1.1 TBq/mmol) or [1-^{3}H]ethan-1-ol-2-amine (0.18–1.1 TBq/mmol) (Amersham, England). Labeling with [^{3}H]ethanolamine was done in HEPES-buffered saline containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1% BSA for 16 h in Falcon culture flasks, and then the cells were washed by gentle centrifugation (800 rpm for 5 min) and incubated at 4 × 10^6 cells/mL in Eppendorf low-absorption centrifuge tubes for an additional period of 1–6 h at 37 °C in the absence (controls) or presence of 5 μg of anti-E2 mAb (O662, IgG3). For [^{3}H]serine labeling, the cells were incubated for 4 h in the conditions described for ethanolamine labeling and then placed in complete culture medium for an additional period of 24 or 48 h to allow the newly synthesized PtdSer to be transferred to the plasma membrane.

Incubations with the mAbs were performed in HEPES-buffered saline containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% BSA, and 2.5 mM glucose for 1–6 h. At the end of this incubation period, the cells were allowed to react with 1.5 mM trinitrobenzenesulfonate (TNBS) (Sigma Chemical Co., Saint Quentin Fallavier, France) for 5 min at room temperature at pH 8.0 according to Gordesky et al. (1975), Bonsall and Hung (1971), and Gordesky and Marinetti (1973). After rapid centrifugation (8000 rpm for 5 s) in an Eppendorf centrifuge, cellular phospholipids were extracted according to Bligh and Dyer (1959) and then submitted to thin-layer chromatography (tlc) on silica gel plates LK6D (Whathman) in the solvent system, (chloroform/methanol/acetic acid/water) (75:45:12:3, v/v), in the presence of phospholipid standards (detected after migration by exposure to iodine vapors). This solvent system allowed the separation of phosphatidylserine (R_f = 0.63) from phosphatidylethanolamine (R_f = 0.89) and TNBS-reacted phospholipids that migrated close to the solvent front. A 5-min time period was chosen on the basis of TNBS reactivity in both control and anti-E2-treated cells. The kinetics of TNBS treatment (Figure 1) indicated that, in our experimental conditions, after a 10-min reaction time, TNBS started to react with control cells. Radioactivity in the different spots was determined with the use of a Berthold Tracemaster 20 automatic tlc analyzer equipped with a 8-mm window and quantified using the integration software supplied with the analyzer.

Labeling with Merocyanine 540. Human thymocytes or Jurkat cells were incubated for 4 h in the absence or presence of anti-E2 mAbs, either O662 (IgG3) or L129 (IgM), or in the presence of different mAbs used as controls. The different mAbs were X35 (IgG2), a CD3 mAb that induces T cell activation, GT2 (IgG1), D66 (IgM), X11 (IgG1), and O275 (IgG2a), four CD2 mAbs which were used either alone at 5 μg/mL or in pairs (5 μg/mL each) since pairs of CD2 have been shown to deliver activating signals to T cells. Phytohemagglutinin (PHAp) was also used at 2 μg/mL, since this lectin induces both an activating signal and cell aggregation. After incubation with these different mAbs for 4 h, the cells were centrifuged (800*g* for 5 min), resuspended in the buffer described above, and then allowed to react with merocyanine

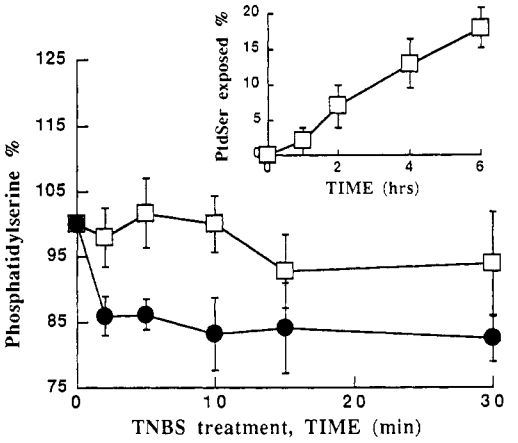

FIGURE 1: Reactivity of TNBS on control (□) and O662 mAb-treated Jurkat T cells (■). Cells were treated for 4 h with O662 and then for different period of time with TNBS, as described in the Materials and Methods section. Unreactive PtdSer was quantified after lipid extraction and thin-layer chromatography. Radioactivity was determined by the use of a Berthold Analyzer. The inset represents the percentage of PtdSer exposed at the cell surface as a function of time after O662 treatment and after 5 min of derivatization with TNBS (since both PtdSer and PtdEtn reacted with TNBS, PtdSer exposed at the cell surface was calculated by subtracting TNBS-unreactive PtdSer from O662-treated cells from PtdSer in control cells).

540 (MC540), a probe of membrane lipid organization sensitive to lipid packing (Williamson et al., 1983; Del Buono et al., 1986). Labeling of cells with MC540 was performed exactly as described by McEvoy and co-workers (1988). The cells were analyzed immediately after the 3-min labeling period with the use of a flow cytometer (FACStar+, Becton Dickinson). The laser was tuned to 514 nm, and fluorescence was monitored through a 560-nm dichroic filter. Results are expressed as the intensity of fluorescence in arbitrary units. Basal fluorescence measured on untreated control cells was subtracted in each experimental condition.

Detection of Phosphatidylserine by Cytofluorimetry. Cells, either Jurkat T cells, human T lymphocytes, or human thymocytes, at 2 × 10^6/mL were incubated for 4 h in the presence of either O662 or control mAbs, washed three times by gentle centrifugation in PBS (pH 7.4), and then incubated for 1 h in the presence of either nonimmune rabbit serum (control) or rabbit anti-PtdSer (Immunotech, Marseille, France), final concentration 1:1000. The specificity and procedure for obtaining anti-phosphatidylserine antibodies have been previously described (Maneta-Payret et al., 1988, 1989). After three washes with 2 mL of PBS, the anti-PtdSer-labeled cells were detected by using phycoerythrine-labeled goat anti-rabbit Fab'2 (Sigma Chemical Co.). After two washes with PBS, the cells were analyzed on the FACStar+. The laser was tuned to 488 nm, and fluorescence was read at 570 nm. Forward and side scatters were used in order to include damaged cells. In some assays, PtdSer was labeled with anti-PtdSer as described above, but the cells were seeded in 96-well plates (Nunc) and the fluorescence was read on a Cytofluor 2350 (Millipore) equipped with 485 (excitation) and 590 nm (emission) filters.

RESULTS

Effect of anti-E2 on PtdSer and PtdEtn Distribution. Cells, either Jurkat or human thymocytes, were labeled to isotopic equilibrium with either [^{3}H]serine or [^{3}H]ethanolamine for 24 h. After two washes, the labeled cells were incubated for 1–6 h in the presence of the anti-E2 mAb O662 at 10 μg/mL.

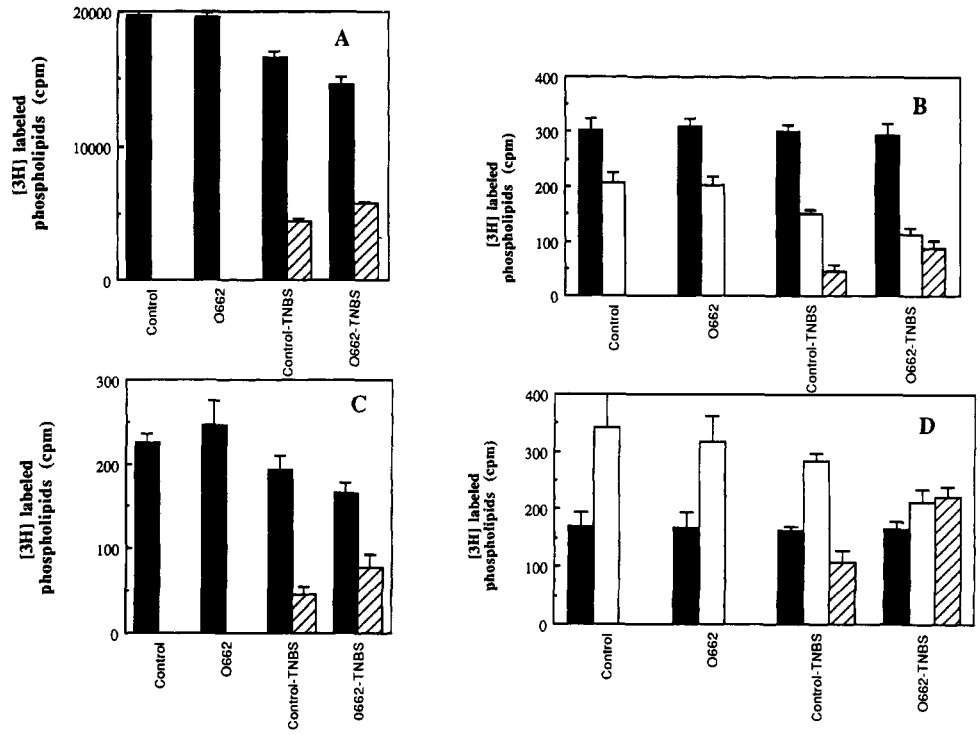

FIGURE 2: Effect of anti-E2 (O662, IgG3) on the translocation of phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) in human thymocytes (A and B) and Jurkat T lymphocytes (C and D) as detected by derivatization with the anionic phospholipid probe, trinitrobenzenesulfonate (TNBS). Cells were metabolically prelabeled for 24 h with either [^{3}H]serine (B and D) or [^{3}H]ethanolamine (A and C). Results are expressed as cpm ± SD (two experiments done in triplicate). Black bars, white bars, and hatched bars represent PtdEtn, PtdSer, and TNBS-derivatized phospholipids, respectively. In panels B and D, the black bars represent the portion of PtdSer transformed into PtdEtn by the decarboxylation pathway.

At the end of this incubation period, the cell phospholipids were derivatized with TNBS as described in the Materials and Methods section, extracted with chloroform/methanol, and submitted to thin-layer chromatography. As shown in Figure 2A, in ethanolamine-labeled cells, O662 induced a small increase in TNBS-derivatized PtdEtn without changing the amount of total [^{3}H]PtdEtn recovered. A similar result was obtained in [^{3}H]ethanolamine prelabeled thymocytes (Figure 2C). When cells were prelabeled with [^{3}H]serine, both PtdSer and PtdEtn arising from decarboxylation of PtdSer could be measured in both Jurkat and thymocytes. TNBS derivatization of phospholipids was markedly enhanced in cells treated with O662 as compared to control cells in both Jurkat (Figure 2B) and thymocytes (Figure 2D). The total amount of labeled phospholipids was not changed after incubation with O662, indicating that this mAb did not modify phospholipid synthesis. This point was further confirmed by directly studying phospholipid synthesis. As shown in Figure 3, O662 had no influence on either [^{3}H]ethanolamine or [^{3}H]-serine incorporation into phospholipids of thymocytes and Jurkat cells (not shown). A similar result (not shown) was observed in thymocytes. The kinetics of O662-induced TNBS reactivity of PtdSer at the cell surface (Figure 1) indicated that the exposure of the phospholipid was significantly enhanced ($p < 0.05$) after 2 h of treatment with the mAb.

The percentages of both PtdSer and PtdEtn exposed at the cell surface in either Jurkat or thymocytes are shown in Figure 4. In untreated control cells, no PtdSer could be derivatized with TNBS, while after treatment with O662 nearly 25% of the PtdSer reacted with the probe. For PtdEtn, 22% and 17% of the phospholipid reacted with TNBS in control Jurkat and thymocytes, respectively. After O662 treatment, 30% and 32% of PtdEtn were reactive with the probe, respectively. This indicates that the portion of PtdEtn that is exposed at the cell surface in basel conditions can be increased after O662 treatment. Other mAbs directed against the CD3 or CD2 molecules were unable to induce exposure of PtdSer in T cells (not shown).

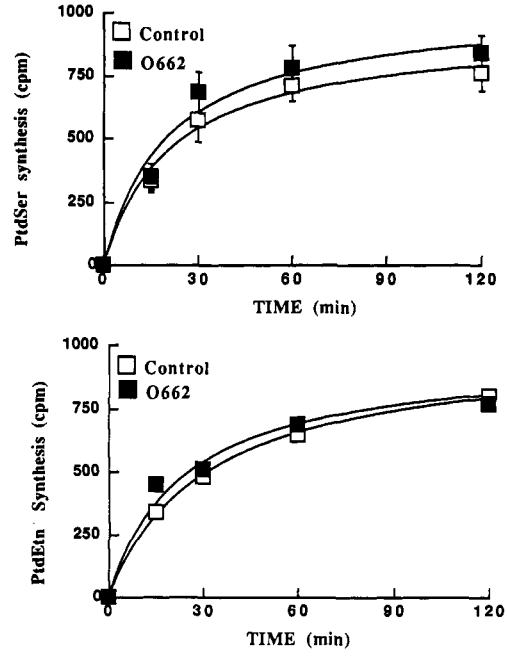

FIGURE 3: Lack of effect of the anti-E2 mAb, O662, on PtdSer and PtdEtn synthesis in human thymocytes. Cells were incubated at time 0 without or with O662 (10 μg/mL) in the presence of 4 μCi of either [^{3}H]serine (A) or [^{3}H]ethanolamine (B). The incorporation of the polar head group into phospholipids was determined after phospholipid extraction and thin-layer chromatography. Results are expressed as cpm ± SD.

Labeling with Merocyanine 540 (MC540). MC540 has been previously described to be a suitable probe to monitor

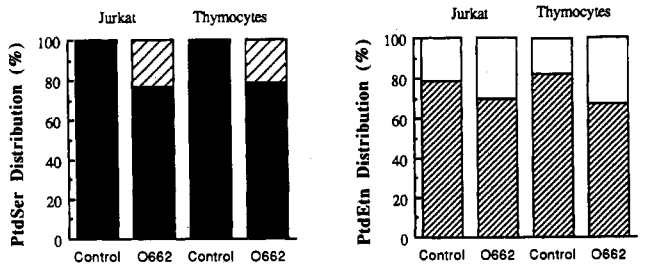

FIGURE 4: Distribution of PtdSer (left) and PtdEtn (right) at the surface of either Jurkat T cells or thymocytes treated for 5 h with 10 μg of O662. The hatched areas in the left panel correspond to the portion of [^{3}H]PtdSer reactive with trinitrobenzenesulfonate, calculated as in Figure 1. The white areas in right panel correspond to the portion of [^{3}H]PtdEtn reactive with the same probe. The experiment was done as described in the legend of Figure 1 and the Materials and Methods section.

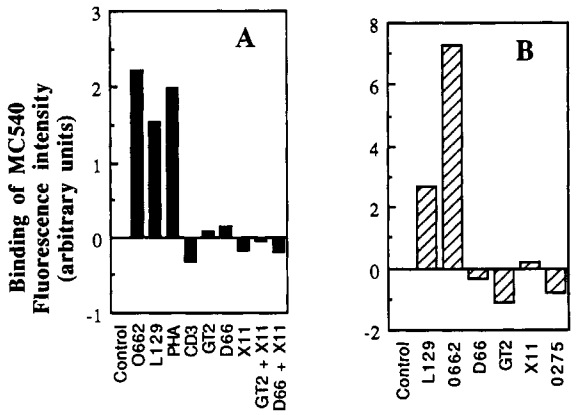

FIGURE 5: anti-E2 mAbs O662 (IgG3) and L129 (IgM) specifically induce labeling of the cell membrane with merocyanine 540 in human thymocytes (B) and Jurkat T cells (A). The results are expressed as the intensity of fluorescence in arbitrary units. Basal fluorescence measured on untreated controls cells was subtracted in each experimental condition. PHA was used at 2 μg/mL and the different mAbs at 5 μg/mL. Cells were treated for 4 h with the effectors and then labeled with MC540 for 3 min and immediately analyzed by cytofluorimetry.

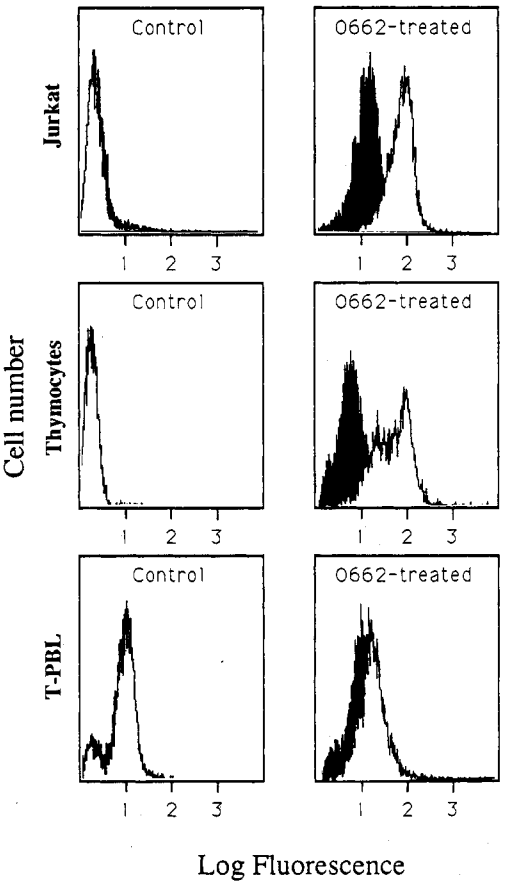

FIGURE 6: anti-E2 mAb O662 induces translocation of phosphatidylserine (PtdSer) in Jurkat T cells and human thymocytes but not in human T cells purified from peripheral blood, as revealed by cell surface labeling with anti-PtdSer antiserum. Jurkat cells (top panels, control or O662-treated for 5 h) were reacted with either nonimmune rabbit serum (black) or anti-PtdSer serum (white). Similar experiments are shown with human thymocytes (control and O662-treated, middle panels) and with human peripheral blood T cells, T-PBL (control and O662-treated, bottom panels). Phycoerythrine-labeled Fab'2 goat anti-rabbit was used to monitor the fluorescence by cytofluorimetry, as described in the Materials and Methods section.

membrane lipid organization (McEvoy et al., 1988) since this dye appears to be highly sensitive to lipid packing (Williamson et al., 1983; Schlegel et al., 1980). Exposure of PtdSer in T lymphocytes undergoing apoptosis has been shown to be accompanied by increased labeling with MC540 (Fadok et al., 1992); we have thus studied whether Jurkat cells and thymocytes treated with anti-E2 mAbs became reactive to this type of probe. Analysis by cytofluorimetry of the control and O662- or L129-treated cells clearly demonstrated that anti-E2 mAbs induced strong incorporation of the fluorescent probe into the membrane both in Jurkat cells (Figure 5A) and in thymocytes (Figure 5B). This labeling with MC540 was specific since other mAbs directed against CD3 or CD2 (GT2, D66, X11, O275) used either alone or in activating pairs were unable to induce the binding of the probe. By contrast, phytohemagglutinin (PHA) caused a high binding of MC540 to Jurkat cells.

Detection of PtdSer Exposed at the Cell Surface by Cytofluorometry with anti-PtdSer Antibodies. Cells, either Jurkat, thymocytes, or human peripheral blood T lymphocytes, were incubated in the absence or presence of O662 for 5 h and then incubated with nonimmune rabbit serum or rabbit anti-PtdSer. The presence of PtdSer at the cell surface was next monitored by using a goat anti-rabbit Fab'2 coupled to phycoerythrin. The results presented in Figure 6 showed that in Jurkat and thymocytes treated with O662, but not in peripheral blood lymphocytes, PtdSer was evident at the cell surface. Labeling with anti-PtdSer was markedly higher than that with control rabbit serum. The specificity of O662 and L129 mAb to induce exposure of PtdSer at the cell surface was tested by using a number of mAbs directed against CD3 and CD2 (used alone or in combination) in order to induce T cell activation. As shown in Figure 7, only O662 and L129 were able to induce exposure of PtdSer (as revealed by using anti-PtdSer antibodies). The specificity of this process was further studied by using another anti-E2 mAb, 12E7, recognizing a different epitope of the E2 molecule. As shown in Figure 7, this mAb was unable to induce exposure of PtdSer in Jurkat cells. Similar results (not shown) were obtained when thymocytes were used in place of Jurkat cells. The kinetics of PtdSer translocation as determined with anti-PtdSer antibodies (Figure 8) indicated that the translocation process was rapid (detectable 1 h after the addition of O662) and developed as a function of time, becoming highly significant ($p < 0.01$) after 4 h of incubation with anti-E2 mAbs. In addition, the kinetics of PtdSer exposure at the cell surface detected by using tritiated precursors of phospholipid synthesis (Figure 1, inset) parallels the kinetics determined with anti-PtdSer antibodies, indicating that the two methods detected the same phenomenon.

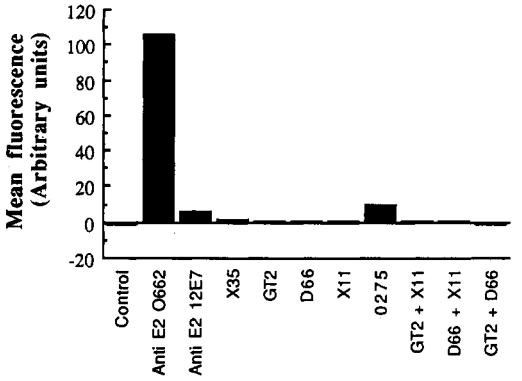

FIGURE 7: Specificity of anti-E2 mAb O662- and L129-induced exposure of PtdSer in Jurkat T cells. Cells were treated for 4 h in the presence of 5 μg/mL of either O662 or mAbs able to activate Jurkat cells (a CD3 mAb, X35, or different CD2 mAbs, D66, GT2, X11, or O275 used alone or in activating combinations, either GT2+X11, D66+X11, or GT2+D66). An anti-E2 mAb, 12E7, directed against an epitope that differs from that recognized by O662 was also included as control. The fluorescence intensity was determined by cytofluorimetry as described in the legend of Figure 5. The fluorescence value of control cells (reacted with rabbit anti-PtdSer followed by Fab'2 anti-rabbit conjugated with phycoerythrin) was subtracted in each condition.

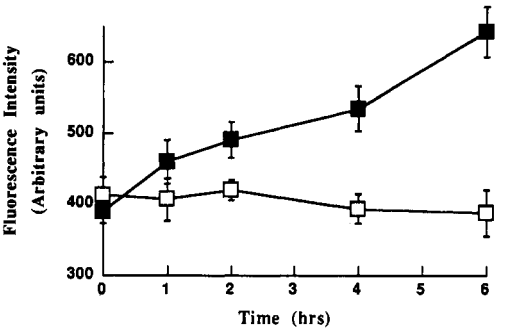

FIGURE 8: Kinetics of the O662-induced appearance of PtdSer at the thymocyte cell surface. Cells were treated with O662 for different periods of time (0–6 h) and then reacted with rabbit anti-PtdSer antibodies and next incubated with goat anti-rabbit Fab'2 coupled to phycoerythrin. Fluorescence was measured in 96-well plates and monitored with a Cytofluor (Millipore) apparatus. Control cells (□) were incubated with anti-PtdSer and a Fab'2-phycoerythrin conjugate. O662-treated cells (■) were incubated with 10 μg/mL mAb for the time indicated. Results are expressed as fluorescence intensity in arbitrary units ± SD (n = 3).

DISCUSSION

E2, the MIC2 gene product, is a 32-kDa protein that is highly glycosylated, the sugar residue accounting for 14 kDa in the molecular mass (Aubrit et al., 1989). This study has revealed that all of the sugar residues are O-linked. The primary structure of E2, deduced from the nucleotide sequence, had shown that E2 displays an organization typical of an integral transmembrane protein (Gelin et al., 1989a). The E2 molecule has a peculiar distribution within T cells. Although all T cell populations tested are reactive with the anti-E2 mAbs, it has been shown that cortical thymocytes expressed high amounts of E2, while medullar thymocytes poorly expressed this molecule. Mature peripheral blood T cells displayed an intermediary surface density. The presence of E2 on numerous T cell lines has also been described (Bernard et al., 1988). The role of E2 in T cell adhesion has been previously described. It has been shown that E2 plays a role in the adhesion of T cells to red blood cells (rosetting) (Bernard et al., 1988; Arbrit et al., 1989), and more recently our group has shown that mAbs directed against E2 induce homotypic aggregation of human thymocytes (manuscript in preparation).

Since cell adhesion as studied with platelets (Rote et al., 1993; Bevers et al., 1989), T lymphocyte–macrophage interactions (Fadok et al., 1992), red blood cell–macrophage interactions (McEvoy et al., 1986), and red blood cell–endothelial cell interactions (Schlegel et al., 1985) appears to involve translocation of PtdSer and, to a lesser extent, PtdEtn, the aim of the present work was to study whether E2-induced aggregation is also accompanied by translocation of these phospholipids. We provide here experimental evidence that O662 and L129, two anti-E2 mAbs that produce cell aggregation of Jurkat cells and thymocytes but not of peripheral blood lymphocytes, provoked translocation of PtdSer and, to a lesser extent, PtdEtn at the surface of both Jurkat cells and thymocytes. Another mAb directed against a different epitope of the E2 molecule, 12E7, had no effect on PtdSer translocation and did not induce thymocyte aggregation. Translocation of PtdSer was not evidenced in peripheral blood T cells, a result that correlates well with the fact that O662 and L129 mAbs did not induce these cells to aggregate. The use of different mAbs directed against the CD3 or CD2 molecules indicates the specificity of the effect induced by O662 and L129 and allows us to conclude that T cell activation is not the cause of PtdSer translocation. This further indicates that changes in intracellular Ca^{2+} concentration induced by CD3 or CD2 mAbs is not responsible for PtdSer translocation.

A role of intracellular Ca^{2+} has been previously illustrated by Williamson et al. (1992) in human erythrocytes treated with 20 μM A23187; the lack of any effect of CD3 or CD2 mAbs probably reflects the fact that the cytosolic Ca^{2+} concentration evoked by the mAbs is largely inferior to that induced by 20 μM Ca^{2+} ionophore. The kinetics of PtdSer translocation (Figure 1 and Figure 8) indicated that this process is very rapid (significantly different from control cells 2 h after the addition of O662 mAb). The appearance of PtdSer at the cell surface is not due to endocytosis since the CD3 mAb that is rapidly internalized in Jurkat cells (Schaffar et al., 1988) does not provoke PtdSer translocation. Recently, Fadok et al. (1992) showed that thymocytes undergoing apoptosis after treatment with dexamethasone expressed PtdSer at the outer leaflet of the plasma membrane. Whether anti-E2 mAbs provoked apoptosis of human thymocytes is not known at this time, but it is important to note that, if this were the case, PtdSer translocation would be one of the earlier events detected during this process.

PtdSer is maintained at the inner leaflet of the plasma membrane by a translocase having a molecular mass of 30–32 kDa (Connor & Schroit, 1988). Despite the analogous mass, the possibility that E2 will be the translocase is unlikely since E2 is present on peripheral blood human T cells and since, in these cells, anti-E2 mAbs did not produce PtdSer translocation. On the other hand, the translocase has been largely characterized (Schroit et al., 1990; Connor & Schroit, 1989; Shroit & Zwaal, 1991) and appears to belong to the family of Rh blood group polypeptides, whereas we, on our side, have shown that E2 is related to the Xga blood group system (Gelin et al., 1989b). If, as suggested, E2 is not the translocase, the translocation of PtdSer induced by anti-E2 mAbs probably means that E2 is associated either with the 32-kDa translocase described by Connor and Schroit (1988) or with the 115–130-kDa Mg^{2+}-ATPase involved in the maintenance of lipid asymmetry [see Devaux (1991) for a review].

The probable relationships between homotypic aggrrgation of thymocytes and PtdSer translocation as hypothesized herein necessitate further work to be firmly established. Nevertheless,

the present work is, to our knowledge, the first demonstrating that the E2 molecule, which has been previously implicated in the adhesive properties of lymphocytes, could be triggered with monoclonal antibodies and induce a marked PtdSer translocation. In addition, together with the recent report of Rote et al. (1993), it appears important to note that anti-PtdSer antibodies, either polyclonal or monoclonal, could be used in conjunction with cytofluorimetry to document the localization of PtdSer in the cell membrane.

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