Aggregation of receptors for immunoglobulin G (FcγRs) on myeloid cells activates a series of events that are key in the inflammatory response and that can ultimately lead to targeted cell killing by antibody-directed cellular cytotoxicity. Generation of lipid-derived proinflammatory mediators is an important component of the integrated cellular response mediated by receptors for the constant region of immunoglobulins (Fc). We have demonstrated previously that, in interferon-γ-primed U937 cells, the high affinity receptor for IgG, FcγRI, is coupled to a novel intracellular signaling pathway that involves the sequential activation of phospholipase D, sphingosine kinase, calcium transients, and protein kinase C isoforms, leading to the activation of the NADPH-oxidative burst. Here, we investigate the nature of the phospholipase that regulates arachidonic acid and eicosanoid production. Our data show that FcγRI coupling promotes iPLA2αβ for the release of arachidonic acid and production of leukotriene B4 and prostaglandin E2. Activation of iPLA2αβ was protein kinase C dependent and platelet-activating factor (PAF) and sphingosine kinase, calcium influx, protein kinase C, calcium transients, and protein kinase C isoforms, leading to the activation of the NADPH-oxidative burst. These studies demonstrate that intracellular signaling via iPLA2αβ plays a pivotal role in the generation of leukotriene B4 and prostaglandin E2.

Receptors for the constant region of immunoglobulin G (IgG) lead to cellular responses, including the internalization of immune complexes, the release of proteases, activation of protein kinases, and the generation of eicosanoids. Receptor aggregation can ultimately lead to targeted cell killing through antibody-directed cellular cytotoxicity (1, 2). These FcγRs, therefore, play critical roles in host defense mechanisms against invading pathogens in autoimmune diseases (3) and in cancer surveillance (4). We have recently reported that, in the human monocyte model (cytokine primed U937 cells), aggregation of the high affinity receptor for IgG (FcγRI) activates, through non-receptor tyrosine kinases, a novel signaling pathway that involves the sequential activation of phosphatidylinositol 3-kinase, phosphatase D, and sphingosine kinase (5–7). This pathway prepares for efficient intracellular trafficking of FcγRI-internalized immune complexes to lysosomes for degradation, the release of calcium from intracellular stores, and the oxidative burst (6–8).

Eicosanoids, characterized by the presence of 20-carbon, unsaturated fatty acid that is hydrolyzed from membrane phospholipids by phospholipase A2 (PLA2) (9). The best studied PLA2s are Groups IIA, V, and IVA, which are highly regulated, Ca2+-dependent enzymes (10, 11). Group IV comprise two native splicing variants, the majority of which are enzymatically functional (12, 13). Mammalian iPLA2αβ are classified as groups VIA and VIB, (iPLA2α and iPLA2β, respectively) (10).

The abbreviations used are: Fc, immunoglobulin constant region receptors; FcγRI, high affinity receptor for IgG; PGE2, prostaglandin E2; LTβ, leukotriene B4; AA, arachidonic acid; PLA2, phospholipase A2; PAF, platelet-activating factor; IFN, interferon; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; TBS, Tris-buffered saline; BELL, E-6-chromomethylene/tetrahydro-3-(1-naphthalenyl)-2-H-pyran-2-one; MAF, methyl arachidonyl fluorophosphate; Bis, bisindolylmaleimide I; CaM, calmodulin.

Retracted
iPLA₂β activation. Although both cPLA₂α and iPLA₂β are expressed in U937 cells, only iPLA₂ for AA release and eicosanoid production through FcγRI is required for the RI-mediated generation of arachidonic acid. Basal iPLA₂β aggregation in FcγRI-transfected U937 cells (lane 1) and IFN-γ-differentiated U937 cells (lane 2) with 200 ng/ml interferon (IFN)-γ activated FcγRI-mediated generation of arachidonic acid; the generation of key inflammatory mediators is triggered by immune-receptors and its functional role is proposed to be the generation of AA and eicosanoids after platelet aggregation in cells pretreated with a specific iPLA₂ inhibitor. Results are the mean of S.D. for triplicate measurements and from three separate experiments.
(50% slurry from Amersham Biosciences) at 4°C, with rocking for 2 h to form precipitating complexes. Cell lysates were precleared with protein A-agarose (incubated for 30 min under rocking conditions); after the removal of the protein A-agarose, the precleared cell extracts were incubated with either anti-iPLA2β or anti-cPLA2α precipitating complexes and placed in a tumbler at 4°C for 4 h, after which the precipitates were washed 3× in ice-cold phosphate-buffered saline to discard unbound material. The precipitated proteins were resolved by SDS-PAGE.

**Gel Electrophoresis and Western Blotting**—Proteins were resolved as described previously (6). Briefly, immunoprecipitates were resolved on 10% polyacrylamide gels (SDS-PAGE) under denaturing conditions and stained with Coomassie blue. The precipitated proteins were resolved by SDS-PAGE.

Measurement of Arachidonic Acid Release—AA release was measured as described previously (25). Briefly, cells were labeled (2 × 10⁶ cells/ml, Amersham Biosciences) in the cell culture medium for 16 h. After washing, the cells were incubated at 37°C for 30 min in RPMI 1640 medium, 1% fetal calf serum containing or not the specific inhibitors. FcγRI was stimulated and reaction stopped at the indicated times. After stimulation, the cells were washed overnight at 4°C with 0.1% Triton X-100 in phosphate-buffered saline. The permeabilized cell suspensions were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (anti-goat IgG-peroxidase conjugate, Sigma) or anti-rabbit IgG-peroxidase conjugate (Sigma) for 3 h at room temperature. The membranes were washed extensively in the washing buffer, and bands were visualized using ECL Western blotting detection system (Amersham Biosciences). On separate experiments, the eluted proteins from the immunoprecipitation were resolved as above, and the gels were subjected to silver staining.

Measurement of PGE2 Synthesis—After receptor stimulation by Biotrak™ PGE2 system from Amersham Biosciences. Briefly, the assay is based on the competition between unlabelled PGE2 and a fixed quantity of peroxidase-labeled PGE2 for a limited number of binding sites on a PGE2-specific antibody. The amount of peroxidase-labeled PGE2 is measured as described previously (25). The PGE2 immunoassay system from Amersham Biosciences is based on the competition between unlabelled PGE2 and PGE2-specific antibody. The amount of PGE2 generated is inversely proportional to the signal generated by the fixed amount of peroxidase-labeled PGE2.

Fluorescence Microscopy—After receptor stimulation by BEL (29), a relatively selective inhibitor for PLA2, the expression of PLA2 isoform(s) was activated by FcγRI, we examined the effect of the PLA2 inhibitors on PAF-mediated AA release. freshly isolated or differentiated cells and in cytokine (IFN-γ) differentiated (Fig. 1b). This FcγRI-triggered AA generation was almost completely inhibited in cells pretreated with 30 μM of methyl arachidonyl fluorophosphate (MAF), an inhibitor of cPLA2 and iPLA2 (16), suggesting the participation of cPLA2 and/or iPLA2 in the AA generation (Fig. 1b). To discern which of the two isoforms was activated by FcγRI, we examined the effect of E-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2-H-pyran-2-one (BEL) (29), a relatively selective inhibitor for iPLA2. The FcγRI-triggered AA release was inhibited in cells pretreated with 10 μM of BEL (Fig. 1c).

Even though the quantity of BEL used has not been shown to inhibit cPLA2 activity (30), we investigated the role of BEL in the AA release triggered by PAF, a stimulant that we knew activates cPLA2 (31). Although 30 μM of MAF inhibited PAF-triggered AA generation from the IFN-γ-primed cells (Fig. 2a), 10 μM of BEL did not have an effect at all (Fig. 2b). Taken together, these data suggest that PAF indeed activates cPLA2, whereas FcγRI couples to iPLA2.

**FcγRI Aggregation Specifically Stimulates iPLA2β**—To gain further insight into the mechanisms of PLA2β, which plays a major role...
in the signaling pathways triggered by FcγRI, we designed specific antisense oligonucleotides against iPLA2 and cPLA2 to knockdown specifically the expression of each enzyme. We have shown previously that U937 cells are sensitive to antisense manipulation (7, 8). IFN-γ-primed cells were treated with antisense oligonucleotides against iPLA2 or cPLA2 to examine the effect of antisense oligonucleotides on the expression levels of iPLA2 and cPLA2 (Fig. 3a). Lane 1, control-untreated cells (lane 2), cells pretreated with antisense against cPLA2 (lane 3), cells pretreated with antisense against iPLA2 (lane 3), cells pretreated with antisense against cPLA2, the antisense against iPLA2, and the antisense against cPLA2. The antisense against iPLA2 and cPLA2 is shown. In cells treated with antisense to iPLA2, there was a substantial reduction in iPLA2 immunoreactivity (80% reduction measured by densitometry), whereas cPLA2 immunoreactivity remained unchanged. Conversely, in cells treated with antisense to cPLA2, there was a reduction in cPLA2 immunoreactivity (85% reduction measured by densitometry), whereas iPLA2 immunoreactivity remained unchanged. Each antisense oligonucleotide, therefore, acted as an internal control for the other.

In cells pretreated with the antisense oligonucleotide to iPLA2, the increase in AA generation, observed after FcγRI aggregation was significantly reduced, compared with the control cells (Fig. 3b). The reduction in the increase after FcγRI aggregation was about 80% in cells treated with antisense iPLA2 compared with control cells and was proportional to the observed reduction in protein expression by Western blot analysis (Fig. 3a). In contrast, treatment of cells with the antisense oligonucleotide to cPLA2 had no effect on the response. Basal, unstimulated control; XL FcγRI, FcγRI aggregation in IFN-γ-primed control cells; XL FcγRI a.s.cPLA2, FcγRI aggregation in cells pretreated with antisense to cPLA2. Results are the mean ± S.D. for triplicate measurements combined from three separate experiments.

The results from these experiments suggest that iPLA2 is essential for AA release and eicosanoid production in FcγRI-mediated signaling pathways.

**Fig. 3.** FcγRI aggregation specifically stimulates iPLA2. (a) Western blot analysis of the effect of antisense oligonucleotides on the expression levels of iPLA2 and cPLA2. Lane 1, control-untreated cells; lane 2, cells pretreated with antisense against cPLA2; lane 3, cells pretreated with antisense against iPLA2. The antisense against iPLA2 (lane 3) showed an 80% reduction in iPLA2 protein expression compared with control-untreated cells (lane 1). The anti-cPLA2 antisense acts as an internal control for iPLA2 because no reduction in iPLA2 levels is observed compared with control-untreated cells (line 2). Typical results from three separate experiments are shown. (b) FcγRI-mediated release of arachidonic acid. Basal, unstimulated control; XL FcγRI, FcγRI aggregation in IFN-γ-primed control cells; XL FcγRI a.s.cPLA2, FcγRI aggregation in cells pretreated with antisense to cPLA2; XL FcγRI a.s.iPLA2, FcγRI aggregation in cells pretreated with antisense to iPLA2. Results are the mean ± S.D. for triplicate measurements combined from three separate experiments. (c) PAF-mediated release of arachidonic acid is dependent upon cPLA2 and not iPLA2. Basal, unstimulated control; XL FcγRI, FcγRI aggregation in IFN-γ-primed control cells; XL FcγRI a.s.cPLA2, FcγRI aggregation in cells pretreated with antisense to cPLA2; XL FcγRI a.s.iPLA2, FcγRI aggregation in cells pretreated with antisense to iPLA2. Results are the mean ± S.D. for triplicate measurements combined from three separate experiments.
oligonucleotide to cPLA2 had no effect on the FcγRI-mediated generation of AA (Fig. 3b). Contrary to the AA generation triggered by FcγRI, AA generation stimulated by PAF was significantly reduced by about 80% in cells pretreated with the antisense oligonucleotide to cPLA2 but not in cells treated with the antisense against iPLA2 (Fig. 3c).

Moreover, by immunoprecipitation and Western blotting, we found that in FcγRI-stimulated cells, iPLA2α is phosphorylated on serine residues (Fig. 4a, upper left panel), whereas cPLA2α is not phosphorylated by FcγRI engagement (Fig. 4b, top panels). Equal protein loading is shown by stripping the blots and reprobing for iPLA2α or cPLA2α (Fig. 4a, upper right and lower right panels). As a control for the iPLA2β immunoprecipitation, a Western blot of cell extracts depleted of iPLA2β is shown (Fig. 4a, lower left panel), as well as a silver-stained gel showing the elution of a single band after immunoprecipitation with the anti-iPLA2β antibody (Fig. 4a, lower right panel). To further establish the specificity of the system, we show that PAF stimulation causes the serine-phosphorylation of cPLA2α (Fig. 4b, lower panels), whereas PAF stimulation does not cause iPLA2β phosphorylation (data not shown). Furthermore, microscopy analysis of the subcellular localization of the different intracellular PLA2 revealed that, after FcγRI aggregation, iPLA2β translocates to the plasma membrane (Fig. 4c), whereas the cytosolic localization of cPLA2α remained unchanged (Fig. 4d). For all fluorescence

Fig. 4. FcγRI aggregation specifically stimulates the phosphorylation and translocation of iPLA2 but not of cPLA2. a, immunoprecipitation of iPLA2 following FcγRI aggregation at the indicated times. Upper left panel, probed with an anti-phospho-serine-specific antibody; upper right panel, probed with a specific anti-iPLA2 antibody; lower left panel, cell extracts after immunoprecipitation, probed with a specific anti-iPLA2 antibody; lower right panel, silver-stained gel after electrophoresis. Lane 1, total cell lysate; lane 2, low speed supernatant after mixing the lysate with the immunoprecipitating complex; lane 3, recovery from flow-through column; lane 4, eluted protein. Typical results from three separate experiments are shown. b, immunoprecipitation of cPLA2 after FcγRI aggregation (upper panels) or after PAF stimulation (lower panels). Stimulations were stopped at the indicated times. Upper left panel, probed with an anti-phospho-serine-specific antibody; upper right panel, probed with a specific anti-cPLA2 antibody; lower left panel, probed with an anti-phospho-serine-specific antibody; lower right panel, probed with a specific anti-cPLA2 antibody. Typical results from three separate experiments are shown. c, fluorescence microscopy of the subcellular localization of iPLA2 in resting cells (left panel) and after FcγRI aggregation for 5 min (right panel). Typical results from three separate experiments are shown. d, fluorescence microscopy of the subcellular localization of cPLA2 in resting cells (left panel) and after FcγRI aggregation for 5 min (right panel). Typical results from three separate experiments are shown.
microscopy experiments, controls were carried out by adding the secondary antibodies to the cells without giving any signals; the antisense treatment did not influence the levels either of FcγRI- or PAF-receptor expression (data not shown). These data strongly suggest that only iPLA₂ is coupled to FcγRI activation.

iPLA₂β Couples FcγRI to the Generation of LTB₄ and PGE₂—As coupling of FcγRI to arachidonic acid release requires iPLA₂β activation, the role of this enzyme in coupling FcγRI to other signaling pathways, such as the production of eicosanoids, was investigated. Reduction in the expression of iPLA₂β by pre-treatment of cells with the antisense oligonucleotide to iPLA₂β resulted in a substantial inhibition of peak LTB₄ and PGE₂ observed after aggregation of FcγRI (Fig. 5, a and b, respectively). However, the antisense to cPLA₂α had no effect on the FcγRI-triggered eicosanoids production (Fig. 5, a and b).

To ensure that the loss of eicosanoid production after FcγRI activation in cells treated with the antisense oligonucleotide to iPLA₂β was a feature of the loss of coupling of the receptor and not some direct effect of the iPLA₂β antisense oligonucleotide on other members of the signaling pathways (such as cyclooxygenases), LTB₄ and PGE₂ were measured after activation of cells with PAF. Addition of PAF to control cells or cells treated with the antisense iPLA₂ resulted in an identical increase in LTB₄ and PGE₂ production (Fig. 5c); on the other hand, in cells pretreated with the antisense to cPLA₂α, eicosanoid production was substantially inhibited (Fig. 5d). These data indicate that,
in cells pretreated with antisense oligonucleotides, the reduction in iPLA2 expression in the generation of eicosanoids. It has been shown that iPLA2 activity indeed may be involved in the FcRγ1-mediated production of arachidonic acid (AA) (18). Here we show that Bis, a selective PKC inhibitor, inhibits AA generation triggered by FcRγ1 (Fig. 6d). In contrast, the AA generation triggered by PAF was not affected by the PKC inhibitor (Fig. 6a), but it was Substance P- or calcium-dependent (32, 33) and even, at least in one case (where iPLA2 was not activated), intracellular calcium depletion prevented the generation of AA (although in this case (18), the authors suggested that this result was due to the inhibition of a calcium-dependent PKC). Here we show that chelating intracellular calcium with BAPTA had no significant effect on iPLA2 translocation (Fig. 7a), iPLA2 phosphorylation (Fig. 7b), or on AA release triggered by FcRγ1 (Fig. 7c), whereas the BAPTA treatment did indeed block the PAF-induced AA release in the same cells. These data correlate with our previous findings that FcRγ1 triggers calcium-independent PKC activities (32, 33) and suggest that calcium-independent PKC(s) may be involved in triggering iPLA2β after FcRγ1 aggregation in human monocytes. DISCUSSION The two forms of cytosolic PLA2 (cPLA2 and iPLA2) are expressed in U937 cells (26, 27), and we found that differentiation with IFN-γ does not significantly alter the expression levels of either of the two enzymes. Our aim was to find out...
which PLA₂ was involved in the FcγRI intracellular signaling cascades leading to the generation of eicosanoids. In this study, we demonstrated that FcγRI is functionally coupled to iPLA₂β, and that this enzyme is required for FcγRI-mediated generation of arachidonic acid and the formation of leukotrienes and prostaglandins.

iPLA₂β contains a calmodulin (CaM)-binding domain near the C terminus which binds calcium-activated CaM and regulates enzyme activity (34). The binding of CaM to iPLA₂β results in the inhibition of iPLA₂β activity, which is reversible through the removal of Ca₂⁺, and subsequent dissociation of CaM from the C terminus of iPLA₂β (34). Thus, in some models, it is possible for iPLA₂β to be regulated through alterations in cellular calcium ion homeostasis and become activated after dissociation from its complex with Ca⁺²/CaM when intracellular calcium stores are depleted (e.g. by sarcoplasmic reticulum calcium ATPase inhibitors, calcium-ionophores, or agonist stimulation; ref. 35).

Here we report that the FcγRI-triggered AA generation was almost completely inhibited in cells pretreated with MAF, an inhibitor of both cPLA₂ and iPLA₂ (36), suggesting the participation of cPLA₂ and/or iPLA₂ in the AA generation. To discern which of the two isoforms was activated by FcγRI, we examined the effect of BEL, a relatively selective inhibitor for iPLA₂ (29). The FcγRI-triggered AA release was inhibited in cells pretreated with BEL. As a control for the specificity of BEL, we investigated the role of BEL in the AA release triggered by PAF, a stimulant known to activate cPLA₂ (31). We found that, although MAF inhibited the FcγRI-triggered AA generation, treatment of the cells with BEL did not have an effect on the AA release triggered by PAF, showing the selectivity of BEL and suggesting that iPLA₂ was the enzyme involved in the AA release.

Antisense oligonucleotides selectively down-modulate the p62 isoform, suggesting that the iPLA₂β enzyme is required for AA and LTβ release and phosphorylation-dependent calcium-dependent intracellular signaling. Moreover, the PKC inhibitor inhibited the PAF-triggered generation of AA, whereas the MAPK inhibitor had no effect on the AA generation. These results suggest that FcγRI triggers iPLA₂β activation by means of PKC, whereas PAF triggers cPLA₂α via the activation of MAP-kinases. Moreover, the PKC inhibitor also blocked iPLA₂β translocation to the cell periphery and completely blocked the phosphorylation of iPLA₂β that follows FcγRI aggregation. Taking these data together, we suggest that PKC is involved in triggering the activation of iPLA₂β in the FcγRI signaling cascade by phosphorylating and thus promoting the translocation of iPLA₂β to the cell's plasma membrane.

Different stimuli induce AA release in monocytes and macrophages in a Ca²⁺-dependent and phosphorylation-dependent manner because of the activation of cPLA₂ (37, 38). However, PGE₂ generation by zymosan-stimulated macrophages is significantly attenuated by BEL or iPLA₂β antisense (30). Paradoxically, in these cells, iPLA₂β activation seems to be regulated by protein kinase C and is Ca²⁺-dependent, although in this case, the authors (18) suggested that this result was due to a calcium-dependent PKC, which, in turn, activated iPLA₂β. In contrast, other studies have shown ligand-stimulated eicosanoid production in cells that have been treated with calcium chelators such as BAPTA and EDTA (35). In agreement with the latter, we show here that chelating intracellular calcium...
with BAPTA had no significant effect on iPLA_2β translocation, phosphorylation, or AA release triggered by FcyRI, whereas the same BAPTA-AM treatment completely blocked the PAF-induced AA release.

Based upon the effects of BEL, it has been suggested for many years that iPLA_2 mediates AA in different cells stimulated by various agonists (29, 39–42), including during IgG-mediated phagocytosis of human monocytes, where AA release was shown to be triggered in a calcium-independent manner (41, 42). For iPLA_2β, several important signaling functions have been suggested, including its role in agonist-stimulated stimulation of smooth muscle (20) and endothelial cells (21), in lymphocyte proliferation (22), and in endothelium-dependent vascular relaxation (21). Very recently, it was reported that myocardial ischemia activates iPLA_2β in intact myocardium, and that iPLA_2β activation is sufficient to induce malignant ventricular arrhythmias (23). Another recent study shows that functional iPLA_2β is required for activation of store-operated and capacitative Ca^{2+} influx in several cell types (24).

We show here that in a human monocytic cell line, iPLA_2β plays a critical role in the intracellular signaling cascades initiated by the high affinity receptor for IgG (FcyRI) and in its functional role to coordinate the response to antigen stimulation for the production of lipid-derived proinflammatory mediators such as leukotrienes and prostaglandins. These observations strongly suggest iPLA_2β as a potential therapeutic candidate for treating human conditions ranging from ischemia to antigen-mediated inflammatory diseases.

Acknowledgment—We thank A.-K. Fraser-Andrews for editing the manuscript.

REFERENCES
1. Graziano, R. F., and Fanger, M. W. (1967) Immunology 25, 319–330.
2. Fanger, M. W., Shen, L., Graziano, R. F., and Hohlfeld, R. J. (1982) Today 10, 92–99.
3. Hallett, M. D., and Hogarth, P. M. (1991) Immunology Today 12, 447–453.
4. Ely, P., Wallace, P. K., Rambold, G., Harnett, M. M., and Allen, J. M. (1998) Blood 86, 3813–3821.
5. Allen, J. M., and Seed, B. (1999) J. Biol. Chem. 274, 35293–35300.
6. Melendez, A., Floto, R. A., Harnett, M. M., and Allen, J. M. (2001) Blood 98, 3421–3427.
7. Yang, V. W. (1996) Gastroenterology 110, 1224–1233.
8. Six, D. A., and Dennis, E. A. (2005).
9. 11. Balsinde, J., Winnstead, M. V., and Dennis, E. A. (2002) FEBS Lett. 531, 2–6.
10. Larsson, P. K., Claesson, H. E., and Kennedy, B. P. (1998) J. Biol. Chem. 273, 207–214.
11. Dennis, E. A. (1997) Trends. Biochem. Sci. 22, 1–2.
12. Balsinde, J., Balboa, M. A., Insel, P. A., and Dennis, E. A. (1999) Annu. Rev. Pharmacol. Toxicol. 39, 175–189.
13. Murakami, M., and Kudo, I. (2002) J. Biochem. (Tokyo) 131, 285–292.
14. Fitzpatrick, F. A., and Seberman, R. (2001) J. Clin. Invest. 107, 1347–1351.
15. Winnstead, M. W., Balsinde, J., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1488, 28–39.
16. Akiha, S., Misumaga, S., Kume, K., Hayama, M., and Sato, T. (1999) J. Biol. Chem. 274, 19966–19972.
17. Winnstead, M. W., Balsinde, J., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1488, 28–39.
18. Akiha, S., Misumaga, S., Kume, K., Hayama, M., and Sato, T. (1999) J. Biol. Chem. 274, 19966–19972.
19. Winnstead, M. W., Balsinde, J., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1488, 28–39.
20. Akiha, S., Misumaga, S., Kume, K., Hayama, M., and Sato, T. (1999) J. Biol. Chem. 274, 19966–19972.
21. Winnstead, M. W., Balsinde, J., and Dennis, E. A. (2000) Biochim. Biophys. Acta 1488, 28–39.