BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **226,** 82–87 (1996)
ARTICLE NO. 1314

Phosphatase Inhibitors Promote ICAM-1 and LFA-1 Mediated Homotypic Aggregation of Jurkat and U937 Cells

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Received August 2, 1996

Here we investigated the role of cellular phosphatases in activation of homotypic aggregation in leukocytes by adding the phosphatase inhibitors okadaic acid (OA) and calyculin A (CA) to cultures of the human monocytic U937 cell line and the human T-lymphocytic Jurkat cell line. We found that OA produces a 120-fold increase of homotypic aggregation with Jurkat cells and a 200-fold increase with U937 cells. Calyculin A increased aggregation of Jurkat cells 10-fold and U937 cells 88-fold. Monoclonal antibodies to LFA-1 completely inhibited the OA and CA induced aggregation in both cell lines, whereas the monoclonal antibody to ICAM-1 only inhibited U937 cell aggregation. These data suggest that phosphatase activity is important in the regulation of ICAM-1 and LFA-1 mediated homotypic aggregation in leukocytes.

Homotypic aggregation of leukocytes is often observed after their activation. Homotypic aggregation of leukocytes is accomplished through a variety of cell surface receptors including lymphocyte function-associated molecule-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) (1). Phosphorylation of LFA-1 has been shown to be associated with LFA-1 mediated homotypic aggregation (2). These studies have employed phorbol ester protein kinase C activation in order to study homotypic aggregation in leukocytes (2).

Here we used the inhibitors of protein phosphatase 1 and 2A, okadaic acid and calyculin A, to investigate the possibility that homotypic aggregation can result from phosphatase inhibition in addition to kinase activation. Okadaic acid is isolated from the black sponge, *Halichondria okadaic* (3). This protein phosphatase inhibitor results in elevated protein phosphorylation in intact cells (4) by blocking the serine/threonine protein phosphatase pathway (4, 5). Calyculin A is isolated from the marine sponge, *Discodermiuas calyx.* (6). Calyculin A is more specific for protein phosphatase-1 than protein phophatase-2A (6).

In this study we use okadaic acid and calyculin A to investigate the effect of protein phosphatase inhibitors on homotypic aggregation in monocytic U937 cells and lymphocytic Jurkat cells. Furthermore, using monoclonal antibodies to LFA-1 and ICAM-1 we investigate the cell surface receptors which mediate homotypic aggregation in okadaic acid and calyculin A treated cells.

MATERIALS AND METHODS

Materials. Calyculin A (CA), okadaic acid (OA) and methyl okadaate (MOA) were all purchased from LC laboratories (Woburn,MA). Each drug was dissolved in 100% ethanol and stored at −80°C. The original stock concentrations were: 330 μM calyculin A, 370 μM okadaic acid and methyl okadaate. The monoclonal antibodies to LFA-1 (IOT16-CDIIa) and ICAM-1 (anti-ICAM-1 Clone 84H10) were purchased from AMAC (Westbrook, ME).

Cell culture. The human monocytic U937 cell line and human lymphocytic Jurkat cell line were cultured in RPMI

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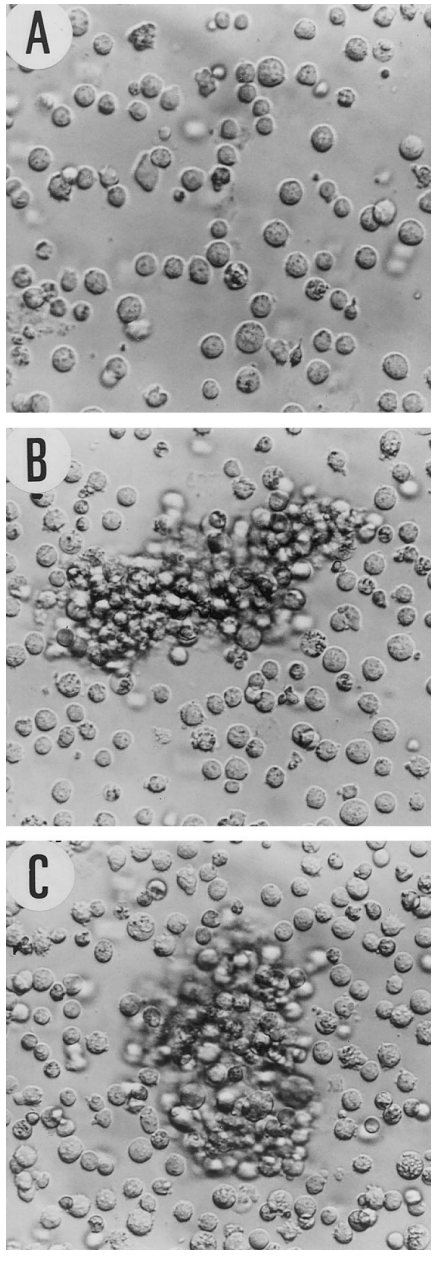

FIG. 1. Phosphatase inhibitors stimulate homotypic aggregation in U937 cells. Untreated U937 cells show no aggregation (A) while U937 cells treated with okadaic acid (B) and calyculin A (C) stimulated form aggregates. Aggregate morphology for okadaic acid treated Jurkat cells were similar to that seen in (B), while the aggregates formed by calyculin A treated Jurkat cells were smaller and fewer (see Fig 3.). Cells were photographed at 20X magnification.

1640 containing 0.1 units/ml of penicillin G sodium and 0.1 μg/ml streptomycin, 10 % fetal calf serum, 2.50 μg/ml amphoterin B and 2.05 μg/ml of sodium deoxycholate in a 37°C CO_2 incubator.

Aggregation assays. Cells were washed three times with serum-free RPMI. 2 $X10^5$ cells were seeded in 0.5 ml of serum free RPMI and added to 16mm diameter wells. OA, MOA and CA were added to the wells at 0 nM, 20 nM,

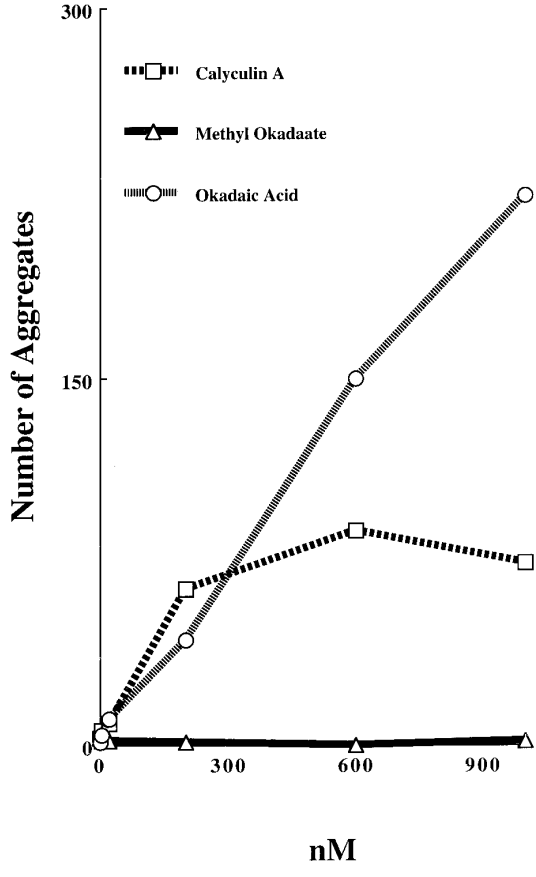

FIG. 2. Aggregation of U937 cells treated with calyculin A (CA), okadaic acid (OA) and methyl okadaate (MOA). Aggregates are defined as 12 or more cells in a single aggregate. The number of aggregates per well was counted at 4X magnification and these numbers are shown above as a measure of aggregation.

200 nM, 600 nM, and 1000 nM. The cells were incubated for six hours at 37°C. After six hours the number of aggregates consisting of 12 or more cells were counted at 4X magnification and this number is presented in figures 2 and 3.

Inhibition of aggregation with LFA-1 and ICAM-1 antibodies. 5.0 X 10_6 cells were seeded in 1 ml of serum-free RPMI and 600 nM of CA or OA in 16 mm diameter wells. Indicated wells received 50 μg/ml of monoclonal antibodies to LFA-1 or ICAM-1. The cells were incubated six hours at 37°C. The aggregation was determined at 20X magnification by counting the number of cells per aggregate and scored as follows; no aggregates (−), 1-5 cells/aggregate (+), 6-10 cells/aggregate (++), 12-20 cells/aggregates (+++), greater than 20 cells/aggregate (++++).

RESULTS

In order to determine the effect of phosphatase inhibition on cell behavior, the phosphatase inhibitors, okadaic acid (OA) and calyculin A (CA), were added to U937 cells. Treatment of cells with various concentrations of OA resulted in a maximum of a 200-fold increase in cell aggregation at 1000 nm OA (Figs. 1 and 2). Treatment with CA resulted in a maximum of an 88-fold increase in aggregation at 600 nM (Fig 2). Methyl okadaate (MOA) is an analog of okadaic acid which does not inhibit phosphatases and was used here as a control. MOA did not stimulate aggregation (Fig. 2). These results show that the inhibition of protein phosphatases stimulates aggregation of U937 monocytic cells

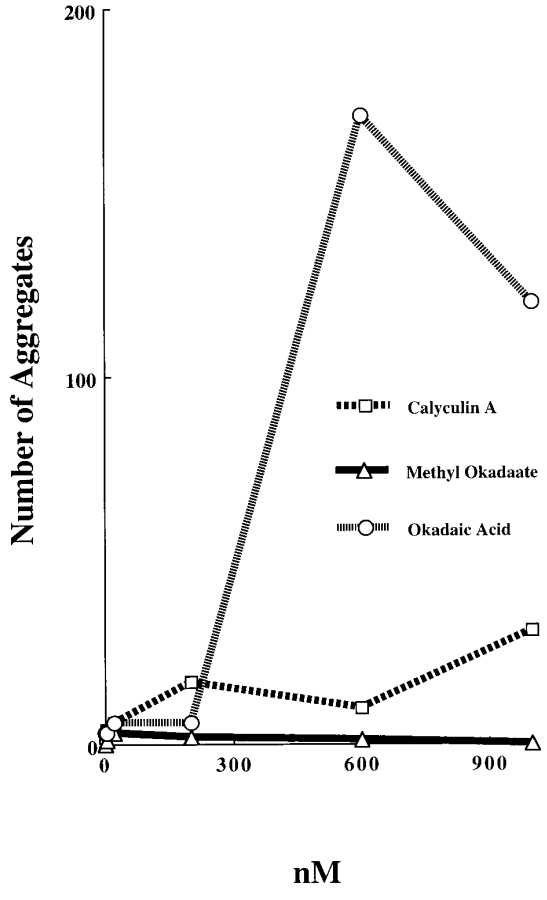

FIG. 3. Aggregation of Jurkat cells in the presence of calyculin A (CA), okadaic acid (OA), and methyl okadaate (MOA). Aggregates are defined as 12 or more cells in a single aggregate. The number of aggregates per well was counted at 4X magnification and these numbers are shown above as a measure of aggregation.

In order to determine the effect of phosphatase inhibition on lymphocytic behavior, the phosphatase inhibitors okadaic acid, (OA) and calyculin A (CA), were added to Jurkat cells. Treatment of cells with OA resulted in a 120-fold increase in cell aggregation. Treatment with CA resulted in an 10-fold increase of aggregation (Fig 3). Methyl okadaate (MOA) had no effect (Fig. 3). These results show that the inhibition of protein phosphatases stimulates aggregation in Jurkat lymphocytic cells.

In order to determine if the leukocyte function-associated molecule-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) play a role in the phosphatase inhibitor-induced aggregation, monoclonal antibodies (mAbs) to LFA-1 and ICAM-1 were added to U937 and Jurkat cells in the presence of OA and CA. The addition of either of these mAbs to U937 cells completely inhibited both CA and OA mediated homotypic aggregation (Table 1). mAb to LFA-1 blocked but CA and OA mediated aggregation of Jurkat cells whereas mAb to ICAM-1 antisera did not inhibit the homotypic aggregation induced by either CA or OA. These results demonstrate that CA and OA treated U937 cells aggregate via LFA-1 and ICAM-1 receptors. Jurkat cell homotypic aggregation after treatment with CA and OA involves LFA-1, but does not appear to require ICAM-1.

TABLE 1

Inhibition of Okadaic Acid and Calyculin A Induced Homotypic Aggregation in Jurkat and U937 Cells by Monoclonal Antibodies to LFA-1 and ICAM-1

	Cell lines	
Treatment	Jurkat	U937
OA no mAb	++	++++
OA anti-LFA-1	−	−
OA anti-ICAM-1	++	−
CA no mAb-	++	++++
CA anti-LFA-1	−	−
CA anti-ICAM-1	++	−

Note. Cells were incubated at 37°C for 3.5 hours in the presence of 600 nM of either okadaic acid (OA), calyculin A (CA), or with no drug additions (blank). In combination with these treatments is the addition of 50 μg/ml of monoclonal antibodies to LFA-1 (anti-LFA-1), or monoclonal antibodies to ICAM-1 (anti-ICAM-1). Aggregation was calculated at 20× magnification by counting the number of cells per aggregate and scored as follows; no aggregates (−), 1−5 cells/aggregate (+), 6−10 cells/aggregate (++), 12−20 cells/aggregate (+++), greater than 20 cells aggregates (++++).

DISCUSSION

Homotypic aggregation has been associated with activation of B-cells, T-cells, monocytes and neutrophiles (7-10). In many cases, homotypic aggregation is mediated at the cell surface by LFA-1 and ICAM-1 interaction (1). Phosphorylation of intracellular domains of LFA-1 stimulates homotypic aggregation and this aggregation can be inhibited by antibodies to either LFA-1 and ICAM-1 (2).

In order to investigate the intracellular mechanisms of homotypic aggregation, a variety of compounds which effect protein phosphorylation have been employed. For example, phorbol ester treatment has been shown to stimulate homotypic aggregation of U937 cell line and the B lymphoblastoid JY cell line (11, 12). Alternatively, calphostin C, an inhibitor of protein kinase C, induces homotypic aggregation in neutrophiles (9). In addition, protein phosphatase 1 and 2A inhibition by okadaic acid has been shown to augment LFA-1 mediated homotypic aggregation in T-lymphocytes (13). In contrast, okadaic acid has no effect on LFA-1 mediated homotypic aggregation in TK1 lymphoma cells (CD8+) (14) and blocks rapamycin induced homotypic aggregation in the YAC-1 T-cell line (15).

Here we show that the serine/threonine phosphatase 1 and 2A inhibitors, okadaic acid and calyculin A, induced homotypic aggregation in U937 and Jurkat cells. While calyculin A is a more potent inhibitor of phosphatase 1 and 2A, this inhibitor was a less effective activator of aggregation than okadaic acid. Indeed with Jurkat cells, little homotypic aggregation was observed with calyculin A compared to okadaic acid. We also show that antibodies to LFA-1 and ICAM-1 were able to inhibit the homotypic aggregation induced by okadaic acid and calyculin A in U937 cells, while a role for ICAM-1 in okadaic acid and calyculin A mediated aggregation in Jurkat cells was not detected. This difference between U937 and Jurkat cells may reflect differences between promonocytes and T-cells, however, it is possible that these differences are restricted to these cell lines. Okadaic acid and calyculin A will be useful tools in future studies which aim to identify the linkage of intracellular signaling pathways to cell

surface adhesion molecules through which various stimuli activate homotypic aggregation in leukocyte populations.

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