Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) Inhibits Low Density Lipoprotein-induced Signaling in Platelets*

Received for publication, December 12, 2002, and in revised form, May 9, 2003
Published, JBC Papers in Press, May 29, 2003, DOI 10.1074/jbc.M212675200

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At physiological concentrations, low density lipoprotein (LDL) increases the sensitivity of platelets to aggregation- and secretion-inducing agents without acting as an independent activator of platelet functions. LDL sensitizes platelets by inducing a transient activation of p38MAPK, a Ser/Thr kinase that is activated by the simultaneous phosphorylation of Thr180 and Tyr182 and is an upstream regulator of cytosolic phospholipase A2 (cPLA2). A similar transient phosphorylation of p38MAPK is induced by a peptide mimicking amino acids 3359–3369 in apoB100 called the B-site. Here we report that the transient nature of p38MAPK activation is caused by platelet endothelial cell adhesion molecule 1 (PECAM-1), a receptor with an immunoreceptor tyrosine-based inhibitory motif. PECAM-1 activation by cross-linking induces tyrosine phosphorylation of PECAM-1 and a fall in phosphorylated p38MAPK and cPLA2. Interestingly, LDL and the B-site peptide also induce tyrosine phosphorylation of PECAM-1, and studies with immunoprecipitates indicate the involvement of c-Src. Inhibition of the Ser/Thr phosphatases PP1/PP2A (okadaic acid) makes the transient p38MAPK activation by LDL and the B-site peptide persistent. Inhibition of Tyr-phosphatases (vanadate) increases Tyr-phosphorylated PECAM-1 and blocks the activation of p38MAPK. Together, these findings suggest that, following a first phase in which LDL, through its B-site, phosphorylates and thereby activates p38MAPK, a second phase is initiated in which LDL activates PECAM-1 and induces dephosphorylation of p38MAPK via activation of the Ser/Thr phosphatases PP1/PP2A.

The contact between platelets and low density lipoprotein (LDL) particles is known to enhance their responsiveness to aggregation- and secretion-inducing agents (1–5). Part of this sensitization is mediated via activation of p38MAPK and cytosolic phospholipase A2 (cPLA2), which, together with a second stimulus, leads to more liberation of arachidonic acid and the formation of thromboxane A2 (1, 6). A second mechanism involved in sensitization is the activation of p125 focal adhesion kinase (FAK) with its still poorly characterized effects on the formation of focal adhesions and cytoskeletal rearrangements (7, 8). Activation of p38MAPK by LDL is rapid (within 10 s at 1 gram/liter LDL) and occurs at LDL concentrations within the physiological range (0.6–1 gram/liter LDL), suggesting that it may affect platelets in the circulation during diet-induced changes in the lipoprotein profile. p38MAPK is a member of the family of proline-directed serine/threonine kinases that is activated by the simultaneous phosphorylation of Thr180 and Tyr182 (9, 10). LDL-induced p38MAPK activation is insensitive to many inhibitors of signal transduction in platelets including the Ca2+ chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA), suggesting that it is an early step in the activation cascade initiated by LDL (6). An exception is an increase in cAMP, which is a potent inhibitor of p38MAPK activation (6).

The receptor through which LDL initiates p38MAPK and FAK activation in platelets has not yet been characterized but is probably not identical to the classical apoB/E receptor that mediates LDL uptake in fibroblasts and smooth muscle cells, because an antibody directed against the ligand binding domain of the classical LDL-receptor does not change the binding of LDL to platelets (11, 12). Furthermore, LDL binding to platelets is normal in platelets from patients with familial hypercholesterolemia who lack the apoB/E receptor (11).

We have recently identified an activating domain in LDL that induces p38MAPK activation in platelets (13). The major apolipoprotein of LDL is apoB100, a 4563-amino acid-long protein that is wrapped around the lipid particle and possesses a recognition site for the apoB/E receptor in the so called B-site (14). A B-site peptide mimetic consisting of 11 amino-acids with a strong positive charge induced a rapid phosphorylation of p38MAPK reaching a peak value after 30 s and returning to pre-stimulation values 5–10 min later (13). The extent of p38MAPK activation was in the range found with 1 gram/liter LDL, and the sensitivity to inhibitors of the signaling steps also resembled that of LDL. This is an important observation, because variations between donors, a long isolation procedure, and, specifically, its susceptibility to oxidative modification have led to conflicting interpretations with respect to the platelet-activating properties of LDL (15).

Because platelet sensitization by LDL might be one of the factors that contribute to the development of thrombo-atherosclerotic disease, its down-regulation is equally important as it prevents persistent platelet sensitization. At present, there is little insight into this mechanism. The presence of platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) is of

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specific interest because its cytoplasmic tail contains a so-called immunoreceptor tyrosine-based inhibitor motif (ITIM), which is a characteristic of receptors that mediate inhibitory signals (16–18). The ITIM family comprises several members such as PECAM-1, FeRRIIB, signal-regulating protein, CD22, and killer inhibitory receptor; of these, only PECAM-1 is present on human platelets (16, 19). PECAM-1 is a 190-kDa transmembrane glycoprotein of the immunoglobulin superfamily of cell adhesion molecules (20). Its 574-amino acid extracellular domain is organized into six Ig-like homology domains (21). There is a single transmembrane domain and a 118-amino acid cytoplasmic tail. The ITIM motif is characterized by the consensus sequence (L/V)I(VS/I)XXX(L/V) (16). The expression of PECAM-1 is restricted to hematopoietic and vascular cell types such as platelets, monocytes, neutrophils, and endothelial cells (20). The functions of PECAM-1 are diverse and include roles in angiogenesis, vasculogenesis, integrin regulation, and the transendothelial migration of leukocytes (22). The activation of PECAM-1 is accompanied by phosphorylation of the cytoplasmic part of the molecule. The cytoplasmic tail of PECAM-1 contains twelve serine, four threonine, and five tyrosine residues (20). Which of these residues are phosphorylated depends on the type of agonist. Both resting and thrombin receptor activating peptide (TRAP)-stimulated platelets show predominantly serine phosphorylation of PECAM-1, provided that the cells do not aggregate. When suspensions are stirred and aggregates are formed, PECAM-1 becomes tyrosine-phosphorylated (23). The contact of platelets with collagen leads to strong PECAM-1 phosphorylation on tyrosine residues (24). A second means to activate PECAM-1 in platelets is by cross-linking with the specific antibody PECAM-1.3, which results in tyrosine phosphorylation of the cytoplasmic tail. Upon receptor activation, the cytoplasmic ITIM-motif recruits and activates a Src-homology 2 (SH2) domain containing protein-tyrosine phosphatases such as SHP-1 and SHP-2 (25). Both SHP-1 and SHP-2 are essential components in PECAM-1-mediated generation of inhibitory signals, and their recruitment and activation depend on the phosphorylation of tyrosine residues on the PECAM-1 cytoplasmic tail (26).

Earlier studies have shown that p38MAPK activation by the B-site in LDL is maximal after 1–2 min and is followed by a gradual decrease to pre-activation values. In the present study we investigated whether PECAM-1 takes part in the down-regulation of LDL-induced p38MAPK activation. Furthermore, we determined whether the down-regulation of p38MAPK activation was mediated by the action of phosphatases.

EXPERIMENTAL PROCEDURES

Antibodies and Reagents—The monoclonal antibody PECAM-1.3 and the polyclonal antibody SEW, both directed against PECAM-1, were kindly provided by Prof. P. J. Newman (The Blood Center of Southeastern Wisconsin, Milwaukee, WI). Anti-mouse F(ab′)2 fragments were from Southern Biotechnology Associates, Birmingham, AL. The goat polyclonal anti-PECAM-1 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibody IV.3 was purified from the monoclonal antibody 4-4B-3C. Immune complexes were detected by enhanced chemiluminescence Western blot reagent was from PerkinElmer Life Sciences. Non-fat dry milk was obtained from Nutricia (Zoetermeer, The Netherlands). PP1 was obtained from Alexis Biochemicals (San Diego, CA). Okadaic acid was obtained from Calbiochem, and vanadate was from Sigma. Human α-thrombin was purchased from Kordia Life Science (Leiden, The Netherlands).

The peptide RLRTRKGLKLA (molecular weight, 1311), designated the B-site peptide, represents the apoB receptor-binding domain (Arg3359 to Ala3369) of apoB100. The peptide was synthesized by standard solid-phase peptide synthesis and purified by C18 reverse-phase chromatography (HPLC, Genosphere Biotechnologies, Paris, France). The purity of the peptide was >99% as determined by high pressure liquid chromatography, and the molecular weight was verified by matrix-assisted laser desorption mass spectrometry by the manufacturer.

Lipoprotein Isolation—Lipoproteins were isolated as described previously (27). In short, fresh, non-frozen plasma from four healthy subjects each containing less than 100 mg of lipoprotein(a) per liter was pooled, and LDL (density range, 1.019–1.063 g/ml) was isolated by sequential flotation in a Beckman L-70 ultracentrifuge. Centrifugations (20 h; 17,5000 × g; 10 °C) were carried out in the presence of NaN3 and EDTA. The LDL preparations contained only minimal amounts of thio-barbituric acid reactive substances (TBARS) (0.20 ± 0.07 mmol/mg), lipid peroxides (6.7 ± 1.9 mmol/mg), and contaminating plasma proteins (below or within reported values for native LDL). Lp(a) concentrations, determined with the use of a specific antibody (Apotech, Organon Technika), were below 14 mg/liter. Lipoproteins were stored at 4 °C under nitrogen for no longer than 14 days, and before each experiment they were dialyzed overnight against 10 volumes of 150 mmol/liter NaCl. ApoB100 and lipoprotein(a) concentrations were measured using the C600 Nephelometer 100. The concentration of LDL was expressed as grams of apoB100 protein per liter.

Platelet Isolation—Freshly drawn venous blood from healthy volunteers was collected with informed consent into 130 mmol/liter trisodium citrate (1:10, v/v). The donors claimed not to have taken any medication during the 2 weeks prior to blood collection. Platelet-rich plasma was prepared by centrifugation (200 × g for 15 min; 20 °C). Gel-filtered platelets were isolated by gel filtration through Sepharose 2B equilibrated in Ca2+ -free Tyrode’s solution (137 mmol/liter NaCl, 2.68 mmol/liter KCl, 0.42 mmol/liter NaHPO4, 1.7 mmol/liter MgCl2, and 11.9 mmol/liter NaHCO3, pH 7.25) containing 0.2% bovine serum albumin and 5 mmol/liter glucose. Gel-filtered platelets were adjusted to a final count of 2 × 108 platelets/liter and incubated with LDL and other agonists (with or without stirring (900 rev/min) at 37 °C as indicated under “Results.”

Measurement of p38MAPK and cPLA2—Gel-filtered platelets were incubated at 37 °C with LDL, B-site peptide, or thrombin as indicated. After incubation, 100-μl aliquots were mixed (1:10, v/v) with cold lysis buffer (radioimmune precipitation assay buffer containing 10% protease inhibitor mixture and 5 mM Na3VO4) and subsequently taken up in Laemmli sample buffer. Samples were heated prior to SDS-PAGE (12%). Proteins were electrophoretically transferred (1 h; 100 volts) to a nitrocellulose membrane using a mini-protein system (Bio-Rad). The blots were blocked in 5% nonfat dry milk and 0.1% Tween 20 in phosphate-buffered saline (1 h; 4 °C). After blocking, the blots were incubated with the PhosphoPlus p38MAPK (Thr180/Tyr182) or p38MAPK antibody (1:2000) and horseradish peroxidase-labeled antibody (1:5000) in nonfat dry milk and 0.1% Tween in PBS; 16 h; 4 °C). Both antibodies are raised against residues 171–186 of human p38MAPK. After washing, the membranes were incubated with horseradish peroxidase-labeled anti-rabbit (1:2000; 1 h; 4 °C), and p38MAPK was visualized using the enhanced chemiluminescence reaction. For semi-quantitative determination of the amount of dual phosphorylated or total p38MAPK, the density of the bands was analyzed using ImageQuant software (Amersham Biosciences).

For the measurement of cPLA2 phosphorylation, samples were withdrawn and collected in Laemmli sample buffer. Measurement of cPLA2 was based on the mobility shift on SDS-PAGE that accompanies phosphorylation of the protein (28). The running buffer for electrophoresis in the presence of cPLA2 was pH 8.0. A monoclonal antibody 4B3 was detected using the monoclonal antibody 4-4B-3C. Immune complexes were detected by enhanced chemiluminescence.

PECAM-1 Tyrosine Phosphorylation and Coprecipitation with PFP2—Platelets were incubated with LDL, the B-site peptide, or thrombin as indicated (37 °C). After incubation, 500-μl aliquots were mixed (1:10, v/v) as described above and precipitated with goat polyclonal anti-PECAM-1 (1 μg) and protein G-Sepharose for 3 h (4 °C). After washing with lysis buffer, samples were taken up in Laemmli sample buffer. Samples were heated (5 min; 100 °C) prior to SDS-polyacrylamide gel electrophoresis (7.5%) and the Western blotting procedure. The blots were blocked in 5% BSA and 0.05% Tween 20 in phosphate-buffered saline (1 h; 4 °C) and then probed with 1 μg/ml of monoclonal 4G10 antibody (1:2000) and polyclonal SEW antibody (1:2000 in 1% BSA and 0.05% Tween 20 in TBS; 16 h; 4 °C). Co-immunoprecipitation of PP2 was determined by incubating the blots with the anti-PFP2 antibody (1:
1000 in 1% BSA and 0.05% Tween in TBS; 16 h; 4 °C). After washing, the membranes were incubated with peroxidase-linked anti-mouse (1:5000; 1 h; 4 °C) or anti-rabbit IgG (1:10000; 1 h; 4 °C), and phosphorylation was visualized using the enhanced chemiluminescence reaction.

Statistics—Data are expressed as means ± S.D. with the number of observations (n).

RESULTS

PECAM-1 Inhibits LDL-induced Phosphorylation of p38MAPK—LDL (1 gram/liter) induced a rapid phosphorylation of p38 MAPK, reaching a peak value after 30 s and returning to pre-stimulation values after 10–20 min (Fig. 1A, left panel). Thus, following a first phosphorylation step, phosphatases were activated that brought p38 MAPK back to the range found in resting platelets. This dephosphorylation of p38 MAPK is distinctly faster than that observed with LDL preparations isolated in the presence of the anti-bacterial agent thimerosal, which was commonly used in earlier studies (6, 29). Thus, following an initial phase in which p38 MAPK was phosphorylated, both LDL and a peptide mimic of the B-site of apoB100 (13) induced dephosphorylation of p38 MAPK, bringing the enzyme back to the state found in resting platelets.

To investigate whether PECAM-1 played a role in the dephosphorylation of p38 MAPK, the receptor was activated with the antibody PECAM-1.3, which is specific for the ectodomain of PECAM-1. Incubation of platelets with this antibody (1 µg/ml) for 10 min resulted in tyrosine phosphorylation of PECAM-1, illustrating that the receptor was activated (Fig. 1A, right panel). No further activation of PECAM-1 was found by cross-linking the PECAM-1.3 antibody with anti-mouse Fab fragments.

When platelets were first treated with the PECAM-1.3 antibody for 10 min and thereafter stimulated with LDL (1 gram/liter) for 1 min, the phosphorylation of p38 MAPK was reduced to 70% compared with platelets that were not treated with the PECAM-1.3 antibody (Fig. 1B). A further cross-linking of PECAM-1 resulted in a reduction in phosphorylation of p38 MAPK to 40%. A similar inhibition by PECAM was observed at the level of cPLA2, which is a downstream target of p38 MAPK in LDL-induced signaling (6).

To assess a possible involvement of the FcγRIIa receptor-mediated pathway in the anti-PECAM-1.3-induced activation of PECAM-1 and the concomitant dephosphorylation of p38 MAPK, studies were repeated in the presence of antibody IV.3, an inhibitor of FcγRIIa (data not shown). The inhibition by PECAM-1 activation was unchanged. This observation, together with literature data that show that p38 MAPK is activated by FcγRIIa activation rather than inhibited by it illustrate that...
the inhibition by PECAM of LDL-induced p38MAPK phosphorylation is independent of FcγRIIa.

**LDL Activates PECAM-1**—The observation that PECAM inhibited the LDL-induced phosphorylation of p38MAPK raised the question of whether LDL itself was capable of activating PECAM-1, thereby down-regulating the initial activation of p38MAPK-activating pathways. Platelets were incubated with LDL, and the phosphorylation of PECAM-1 was visualized on Western blots after immunoprecipitation of the receptor (Fig. 2A). As a control, platelets were stimulated with thrombin (0.5 units/ml; 2 min; 37 °C) with and without stirring. Stirring resulted in an enhanced tyrosine phosphorylation of PECAM-1, whereas serine phosphorylation was equal under both conditions, which was in agreement with earlier observations (23). Western blots with an appropriate antibody revealed that LDL induced tyrosine phosphorylation of PECAM-1 but was incapable of inducing phosphorylation of serine residues. The LDL (1 gram/liter)-induced tyrosine phosphorylation of PECAM-1 was transient, showing a maximum after 1 min of stimulation and down-regulation after prolonged incubation (Fig. 2B). Also, the B-site peptide induced tyrosine phosphorylation of PECAM-1, which is in line with the concept that the B-site mediates the activation of PECAM-1 by LDL. After immunoprecipitation of PECAM-1, an additional band at ~125 kDa was co-precipitated. Repробing of the blots with the polyclonal anti-PECAM-1 (SEW) and an antibody against focal adhesion kinase revealed that the upper band represents PECAM-1 and the lower band c-Src, indicating that a complex was formed between c-Src and PECAM-1 upon stimulation by LDL.

**Down-regulation of LDL Signaling by PECAM-1 Depends on Serine/Threonine Phosphatases**—PECAM-1 is known to initiate inhibitory signaling pathways by recruitment of the serine/threonine phosphatases SHP-1 and SHP-2. As p38MAPK is activated upon phosphorylation of both threonine and tyrosine residues, the involvement of serine/threonine phosphatases as well as tyrosine phosphatases was investigated.

To investigate the contribution of serine/threonine phosphatases, platelets were incubated for 0.5 min with 1 µM okta-
daic acid, a concentration known to inhibit the serine/threonine phosphatases PP1 and PP2A (30, 31). This treatment resulted in a strong potentiation of p38MAPK activation by LDL (1 gram/liter) and the B-site peptide (100 μmol/liter) and changed the transient activation into a more sustained activation compared with LDL-stimulated p38MAPK in the absence of the inhibitor (Fig. 3A).

We next investigated whether inhibition of tyrosine phosphatases with vanadate interfered with the phosphorylation state of p38MAPK. Platelets were preincubated for 30 min with 100 μM sodium vanadate. This treatment completely abolished the phosphorylation of p38MAPK by LDL and the B-site peptide (Fig. 3B). Furthermore, the tyrosine phosphorylation of PECAM-1 induced by LDL and the B-site peptide was preserved by vanadate treatment and even increased to levels above the range found in the absence of the inhibitor (Fig. 3C).

A combination of the two inhibitors induced a dual response. During the first 5 min there was complete inhibition of p38MAPK phosphorylation as observed with vanadate alone. Thereafter, a slight recovery became detectable, but the recovery was later and considerably lower than that seen with okadaic acid alone (Fig. 4A). Thus, following a period in which inhibition by vanadate was predominant, a second phase became apparent in which dephosphorylation of p38MAPK by PECAM-1 was opposed by okadaic acid. Concurrent analysis of PECAM-1 phosphorylation showed that, in the period of p38MAPK inhibition, PECAM-1 was maximally phosphorylated. The later recovery of p38MAPK phosphorylation was accompanied by a fall of phosphorylated PECAM-1, which was slightly faster than in the presence of vanadate alone (Fig. 4B).

Because the effect of okadaic acid on the dephosphorylation of p38MAPK suggested the involvement of a phosphatase of the PP1/PP2α type, a possible co-association between PECAM-1 and PP2A was investigated. Stimulation by LDL induced a transient co-association between Tyr-phosphorylated PECAM-1 and PP2A, supporting the conclusion that PECAM-1 regulates dephosphorylation of p38MAPK through PP2A (Fig. 5).

DISCUSSION

ApoB100 is the main protein constituent of LDL and consists of 4563 amino acids wrapped around the lipid particle. The B-site is the domain in apoB100 that binds to the apoB/E receptors on cells that remove plasma cholesterol from the circulation. A peptide that mimics this region between Arg3359 and Ala3369 of apoB100 induced a transient activation of platelet p38MAPK, showing a maximal phosphorylation after 1 min of stimulation (13). Also, LDL induced a rapid and transient phosphorylation of p38MAPK with approximately similar kinetics as observed with the B-site peptide. It is possible that this down-regulation at prolonged incubation times might reflect a protection mechanism that prevents extensive platelet activation in the circulation by LDL.

Here we report that the down-regulation of phosphorylated p38MAPK is mediated by PECAM-1. PECAM-1 activation by a specific receptor cross-linking antibody led to Tyr-phosphoryl-
thesized in the legends to Figs. 1 and 2.

By dual phosphorylation of the p38 MAPK of Thr180 and Tyr182, PECAM-1 also inhibits the phosphorylation of cPLA2, which is required for optimal PECAM-1 activation (32). Activation of PECAM with additional Fab fragments led to a further reduction in the phosphorylation of the enzyme. These observations make PECAM-1 an important inhibitor of LDL-induced p38MAPK phosphorylation. P38MAPK is activated by dual phosphorylation on tyrosine and threonine residues by upstream enzyme termed MKK kinase. Thus, the dephosphorylation of p38MAPK by collagen revealed that PP2A mediates the dephosphorylation, and a similar role might be present in platelets stimulated by LDL (37).

Okadaic acid is an inhibitor of the serine/threonine phosphatase PP1 and PP2A (36). When platelets were preincubated with this inhibitor, the transient phosphorylation of p38MAPK by LDL and the B-site peptide changed into a persistent activation and reached levels far above the maximal phosphorylation observed in untreated platelets. A recent study on p38MAPK phosphorylation by collagen revealed that PP2A mediates the dephosphorylation, and a similar role might be present in platelets stimulated by LDL (37), p38MAPK is activated by dual phosphorylation on tyrosine and threonine residues by dual specific MAPK kinases 3 and 6 (MKK3/6) (38, 39). MKK6 triggers the phosphorylation of the six tyrosine residues with kinetics that differ greatly from those induced by thrombin (35).

Vanadate is an inhibitor of tyrosine phosphatases. When platelets were preincubated with this inhibitor, the transient tyrosine phosphorylation of PECAM-1 was increased and more sustained, enabling the receptor to transmit a stronger inhibitory signal into the cell. Apparently, vanadate did not increase the phosphorylated state of p38MAPK, although the enzyme is phosphorylated on threonine and tyrosine residues. Instead, vanadate treatment completely abolished the increase of phosphorylated p38MAPK by LDL and the B-site peptide. This illustrates the potent inhibitory action of PECAM-1, which, through its strong activation of PP1/PP2A, abolished the accumulation of phosphorylated p38MAPK. Also, when both inhibitors are present the inhibition by vanadate is predominant, and only after prolonged incubation with LDL does the effect of okadaic acid become detectable. Together, these results support the concept that PECAM-1 is a negative regulator of LDL-induced signaling in platelets by activating a phosphatase sensitive to okadaic acid. Indeed, immunoprecipitates reveal co-association with PP2A. Together with the coprecipitation of focal adhesion kinase (Ref. 35 and this study), the Src-kinases Fgr (35), and

![Fig. 4. Combined inhibition of Ser/Thr and Tyr phosphatases during LDL signaling to p38MAPK and PECAM-1.](image)

**FIG. 4.** Combined inhibition of Ser/Thr and Tyr phosphatases during LDL signaling to p38MAPK and PECAM-1. Platelets were incubated (incub) with LDL (1 gram/liter; 37°C) for the indicated time periods in the presence of okadaic acid (O) (1 μM; 30 s) and sodium vanadate (V) (100 μM; 30 min), and dual phosphorylated p38MAPK (p38MAPK-P) (A) and tyrosine phosphorylation of PECAM-1 (PECAM-P) (B) were analyzed. Data are means ± S.D., n = 4. Further details as described in the legends to Figs. 1 and 2.

![Fig. 5. Co-association between PECAM-1 and PP2A.](image)

**FIG. 5.** Co-association between PECAM-1 and PP2A. Platelets were incubated without and with LDL (1 gram/liter; 37°C), and PECAM-1 was immunoprecipitated (IP) from platelet lysates and applied to gel. Following PAGE, blots (WB) were incubated with an antibody against PP2A, an anti-total PECAM antibody as a control for equal lane loading, and 4G10, which detects tyrosine phosphorylated proteins.
c-Src (this study), LDL-induced tyrosine phosphorylation of PECAM appears central in the formation of a complex containing multiple signaling elements.

Signal transduction by PECAM-1 depends on the ITIM motif, which becomes tyrosine phosphorylated. Because PECAM-1 does not appear to be autophosphorylated, a kinase is thought to phosphorylate the cytoplasmic tyrosine residue (40, 41). A candidate responsible for the tyrosine phosphorylation is a member of the Src family tyrosine kinase (34, 41). Immunoprecipitation studies after the incubation of platelets with LDL showed co-precipitation of c-Src with PECAM-1. P38MAPK activation was not affected by the okadaic acid treatment until the platelets had been stimulated with LDL for 1 min or more. This is in accordance with the time-dependent activation of PECAM-1 by LDL and the recruitment of c-Src. This suggests that c-Src recruitment to the cytoplasmic tail of PECAM-1 induces ITIM-tyrosine phosphorylation and the subsequent recruitment of PP2A.

In conclusion, the results reported in this study are best explained by assuming a model in which LDL affects platelet signaling mechanisms in two phases. During the initial phase, LDL binds via its B-site in apoB100 to the putative LDL receptor on platelets, thereby inducing the dual phosphorylation of p38MAPK and activating the enzyme. This step leads to activation of cPLA2, and the formation of thromboxane A2. This mechanism explains why aspirin or related drugs abolish at least in part the sensitization of platelets by LDL. This activation phase is followed by a second phase in which LDL, either directly or bound in a complex with the LDL receptor, activates PECAM-1. This results in recruitment of c-Src and the phosphatases PP1/PP2A, thereby down-regulating the phosphorylation of p38MAPK.

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