Platelet Factor 4 Enhances the Binding of Oxidized Low-density Lipoprotein to Vascular Wall Cells*

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Accumulation of low-density lipoprotein (LDL)-derived cholesterol by macrophages in vessel walls is a pathogenomic feature of atherosclerotic lesions. Platelets contribute to lipid uptake by macrophages through mechanisms that are only partially understood. We have previously shown that platelet factor 4 (PF4) inhibits the binding and degradation of LDL through its receptor, a process that could promote the formation of oxidized LDL (ox-LDL). We have now characterized the effect of PF4 on the binding of ox-LDL to vascular cells and macrophages and on the accumulation of cholesterol esters. PF4 bound to ox-LDL directly and also increased ox-LDL binding to vascular cells and macrophages. PF4 did not stimulate ox-LDL binding to cells that do not synthesize glycosaminoglycans or after enzymatic cleavage of cell surface heparan and chondroitin sulfates. The effect of PF4 on binding ox-LDL was dependent on specific lysine residues in its C terminus. Addition of PF4 also caused a 10-fold increase in the amount of ox-LDL esterified by macrophages. Furthermore, PF4 and ox-LDL co-localize in atherosclerotic lesion, especially in macrophase-derived foam cells. These observations offer a potential mechanism by which platelet activation at sites of vascular injury may promote the accumulation of deleterious lipoproteins and offer a new focus for pharmacological intervention in the development of atherosclerosis.

The development of atherosclerosis has been attributed to an interplay among several factors, including endothelial cell dedifferentiation, adherence and activation of platelets, genetic and acquired hyperlipidemia, oxidation of lipoproteins, infiltration of the vessel wall with macrophages and their conversion to foam cells, and smooth muscle cell proliferation and migration (1). Activated platelets may contribute to the progression of atherosclerosis through diverse pathways, including the release of potent smooth muscle cell mitogens such as platelet-derived growth factor, epidermal growth factor, and transforming growth factor-β (2–6). In accord with these observations, interventions that reduce platelet number and/or inhibit platelet activation retard the formation of atherosclerotic lesions in several experimental systems (7, 8). Platelet activation also promotes the accumulation of low-density lipoproteins (LDL) by macrophage foam cells (for review see Refs. 9–13). The mechanism(s) by which platelets contribute to the accumulation of LDL in the vessel wall are less clear (14).

One potential candidate that might be involved in the accumulation of LDL is platelet factor 4 (PF4), a cationic protein that is released in large amounts when platelets are activated and that is found in atherosclerotic lesions (15, 16). Mature human PF4 is a 70-amino acid, lysine-rich platelet-specific protein that belongs to the CXC (or β) chemokine subfamily, in which the first two of the four conserved cysteine residues are separated by one amino acid residue (17). Human PF4 has been sequenced (18) and cloned (19), and its x-ray crystallographic structure has been defined (20). PF4 is synthesized by megakaryocytes and comprises 2–3% of the total protein in mature platelets (21). When platelets are activated, PF4 is secreted in concentrations approaching 25 μg/ml (4 μM) in the vicinity of the vessel wall (22). PF4 exists as a tetramer with the three β-sheets of each subunit facing inwards and the N and C termini lying on the surface of the molecule. The four lysine-rich C-terminal α-helices form a circumferential band around the tetramer, where they and other cationic residues have been implicated in the binding of heparin (22, 23).

PF4 secreted by platelets binds primarily to endothelial heparan sulfate-rich proteoglycans, inhibiting the activity of anti-thrombin III (23), to the chemokine receptor Duffy on erythrocytes and endothelial cells (24, 25) and to coagulation proteins such as thrombomodulin (26) and activated protein C (27, 28). PF4 also binds to exposed subendothelium (29), where it may function as an inhibitor of endothelial cell proliferation and angiogenesis (30). However, its effects on vessel wall biology and the formation of atherosclerosis are less clear. Although platelet activation promotes the accumulation of LDL by macrophages, PF4 inhibits the uptake of LDL by fibroblasts (31). In an attempt to elucidate the mechanism by which PF4

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may facilitate the incorporation of LDL into cells, we previously reported that PF4 diverts the uptake of LDL from the LDL receptor to the relatively inefficient PG-dependent endocytic pathway, likely increasing its residency time in tissue and its propensity to undergo oxidation (47).

LDL that has not been internalized and degraded is subject to oxidation in the vascular microenvironment (32). Unlike LDL, which is internalized primarily through the LDL receptor, oxidized LDL is internalized by the scavenger receptor and several other, more recently described receptors (33–35). In the present study we extend these studies by showing that, in contrast to LDL, PF4 stimulates the binding of ox-LDL by vascular cells and promotes its esterification. We propose a novel mechanism by which platelet activation may contribute to the development of atherosclerosis through the release of PF4.

**EXPERIMENTAL PROCEDURES**

**Recombinant PF4 and PF4 Variants**—A number of recombinant PF4-like proteins that had been previously expressed and purified from prokaryotic cells and characterized for their heparin affinity were used. These include wild type (WT) PF4 and neutrophil-activating protein-2 (NAP-2), a series of lysine to alanine substitutions in the lysine-rich C terminus of PF4, and chimeric proteins between PF4 and NAP-2 (36). PF4 was radiolabeled with 125I using lactoperoxidase (Sigma) as described (37). Labeled protein was separated from the free iodine using gel filtration (PD-10; Amersham Biosciences), and the protein concentration was determined using Bio-Rad microprotein enzyme-linked immunosorbent assay.

**Cell Culture**—All cell cultures were maintained at 37 °C under 5% CO2. Chinese hamster ovary (CHO) cells lacking xylosyl transferase activity (XT–), and therefore incapable of initiating the synthesis of heparan sulfate or chondroitin sulfate chains, were the kind gifts of Dr. J. Esko, University of California, San Diego, CA (38). WT and mutant CHO cells were grown in Ham’s F-12 media supplemented with fetal calf serum (10%), glutamine (200 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml). Cultured human umbilical vein endothelial cells (HUVEC) and smooth muscle cells were cultured as previously described (39).

**Liprotein Preparation**—125I-LDL was prepared, radiolabeled, and characterized as described (40, 41). Briefly, LDL was isolated by ultrafiltration at d = 1.019–1.63 mg/ml from human plasma containing 1 mg/ml EDTA. The preparation was filtered under sterile conditions, aliquoted into plastic tubes and kept under N2 at 4 °C without exposure to light. LDL was oxidized as described in Ref. 41. Briefly, LDL was dialyzed against phosphate-buffered saline (PBS) at 4 °C to remove EDTA, then diluted with PBS to 0.1 mg of protein per ml prior to oxidation. LDL was incubated with 10 μM CuSO4 at 37 °C for 18 h. LDL oxidation was terminated by adding 25 μM butylated hydroxytoluene and 1 μM EDTA and by refrigerating the LDL samples at 4 °C. The extent of LDL oxidation was analyzed by the thioharbitoric acid-reactive substances assay, which measures malondialdehyde equivalents (42), and by the lipid peroxides assay that analyzes their capacity to convert iodoide to iodine (43). The amount of cholesteryl linoleate hydroperoxides was measured by high pressure liquid chromatography (44). Electrophoretic mobility of the lipid peroxides was performed on 1% agarose gel. The electrophoretic mobility of the lipid peroxides was defined as the difference between total and nonspecific binding. The same cells and conditions were used to measure the binding of 125I-PF4 as were used to determine the binding of 125I-ox-LDL.

In other experiments, the formation of complexes between 125I-PF4 with LDL and with ox-LDL was examined. 125I-rPF4 (25 nm) was mixed with LDL, ox-LDL, or BSA (50 μg/ml) for 30 min at 37 °C in PBS. The mixture was applied to a Bio-spin 30-gel filtration column (Bio-Rad), and the radioactivity in the excluded volume was measured. To examine the binding of 125I-ox-LDL/PF4 complexes to cells, 125I-ox-LDL was incubated with rPF4 or BSA for 30 min at 37 °C in PBS. The complexes were separated on Sephadex G-75. These and all experiments described below were performed in triplicates and were repeated a minimum of three times, unless otherwise noted. All data are presented as mean ± S.D. of the three experiments.

**Confocal Microscopy**—Cells grown on coverslips were incubated in DMEM containing ox-LDL with or without PF4 and supplemented with 10% fetal calf serum for 30 min at 37 °C. The cells were washed three times with PBS, fixed for 40 min with 4% paraformaldehyde and 0.2% Triton X-100 in PBS-BSA buffer for 30 min. The coverslips were overlaid for 20 min with 1% normal horse serum and then incubated for 40 min with anti-ox-LDL antibodies diluted 1:500 with PBS. After four washes with PBS, the cells were stained for 40 min with Alexa 488-labeled goat anti-rabbit antibody (Molecular Probes), washed four times with PBS and mounted in 80% glycerol and 20% PBS supplemented with 3% DABCO (1,4-diazabicyclo-[2,2,2]-octane) as anti-bleaching agent. No staining was seen when either anti-defensin or the secondary antibody was omitted.

Confocal microscopy was performed using a Zeiss LSM 410 confocal laser scanning system attached to the Zeiss axiovert 135 m inverted microscope. The coverslips were mounted under 0.1% soybean albumin and excited with a 25-nW argon laser (488 nm excitation line with 515 nm low pass filter) for the excitation of Alexa 488 green fluorescence. The differential interference contrast images according to Nomarski were collected simultaneously using a transmitted light detector. Autofluorescence of the specimen was set to background level.

**Cholesterol Esterification**—J774 murine macrophages were cultured in DMEM supplemented with 2 mM glutamine and 10% fetal calf serum. The cells were washed, and the medium was replaced with DMEM containing 200 μM native or ox-LDL in the presence or absence of 100 μg PF4. 3H-oleic acid (100 μM) was added in the form of oleate-albumin complex (3:1 molar ratio) for 24 h at 37 °C, and the incorporation of 3H-oleate into cholesterol esters was counted. To do so, the medium was collected, the cells were washed four times with PBS, and 1 ml of absolute ethanol was then added for 2 h. The ethanol extract was separated by thin layer chromatography on silica gels. The bands were identified and isolated, and the radioactivity incorporated into the cholesterol ester fraction was counted.

**Assay of Cholesterol and Protein Content of Cells**—The total and free cholesterol contents of J774 cells were determined using an enzymatic kit (Roche Molecular Biochemicals). For these assays, J774 cells were harvested by scraping into distilled water and processed as described previously (46). J774 protein content was determined by the method of Lowry et al. using BSA as a standard.

**Immunohistochemistry**—The atherosclerotic arteries were obtained anonymously from either surgically removed or autopsy specimens from the Cooperative Human Tissue Network, Eastern division, after approval from the University of Pennsylvania School of Medicine Institutional Review Board. Formalin-fixed, paraffin-embedded 5-μm sections were deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was quenched with 0.5% hydrogen peroxide, and unreactive sites were blocked with 10% goat serum in 1× automation buffer (Biocom Corp.) for 20 min at 37 °C. Slides were then incubated overnight at 4 °C with either rabbit anti-PF4 antibody (1 μg/ml polyclonal affinity-purified antibody from PeproTech), rabbit anti-ox-LDL antibody (1:250 dilution of rabbit serum from CHEMICON International) or normal rabbit serum (negative control). Sections were incubated for 30 min with biotinylated goat-anti-rabbit IgG (Jackson Laboratories, West Grove, PA) diluted 1:200 at 37 °C. Slides were washed and incubated with streptavidin-horseradish peroxidase (Research Genetics) for 30 min at 37 °C and washed, and...
stable diaminobenzine chromogen (Research Genetics) was applied for 5 min at 20 \(^\circ\) C. Slides were counterstained with dilute hematoxylin.

RESULTS

Effect of PF4 on the Binding and Internalization of ox-LDL—We have previously reported that PF4 diverts the uptake of LDL from the LDL receptor to the relatively inefficient PG-dependent endocytic pathway, likely increasing its residency time in tissue and its propensity to undergo oxidation (47). LDL that has not been internalized and degraded is subject to oxidation in the vascular microenvironment (32). Unlike LDL, which is internalized primarily through the LDL receptor, oxidized LDL is internalized by the scavenger receptor and several other, more recently described receptors (33–35).

Therefore, based on its effects on LDL, we examined the effect of PF4 on the uptake of ox-LDL by cells involved in the pathogenesis of atherosclerosis.

In contrast to its inhibitory effect on the binding of LDL to cells (47), PF4 promoted the binding of ox-LDL to cultured endothelial cells and smooth muscle cells 5-fold, and to J774 cells 7-fold (Fig. 1A). Binding of ox-LDL to HUVEC was increased using \(125\)I-recombinant PF4 (25 nM) incubated with ox-LDL, BSA, or LDL (50 nM each) for 30 min at 37 \(^\circ\) C in PBS. The mixture was applied to a Bio-spin 30 gel filtration column, and the radioactivity in the excluded volume was measured. B, complexes between PF4 (25 nM) and \(125\)I-ox-LDL (50 nM) or \(125\)I-ox-LDL alone were isolated by a gel filtration column and incubated with HUVEC in the absence or presence of heparin (0.2 units/ml).

Mechanism of the PF4 Stimulation of ox-LDL Binding—The finding that PF4 has opposing effects on the binding of LDL and ox-LDL suggests that it may interact differently with the two forms of the lipoprotein. To explore this possibility, we examined the binding of \(125\)I-PF4 to LDL or ox-LDL directly using gel filtration. Indeed, ox-LDL, but not WT LDL or BSA, bound radiolabeled PF4 (Fig. 2A). The finding that PF4 has opposing effects on the binding of LDL and ox-LDL suggests that it may interact differently with the two forms of the lipoprotein. To explore this possibility, we examined the binding of \(125\)I-PF4 to LDL or ox-LDL directly using gel filtration. Indeed, ox-LDL, but not WT LDL or BSA, bound radiolabeled PF4 (Fig. 2A). The finding that PF4 has opposing effects on the binding of LDL and ox-LDL suggests that it may interact differently with the two forms of the lipoprotein. To explore this possibility, we examined the binding of \(125\)I-PF4 to LDL or ox-LDL directly using gel filtration. Indeed, ox-LDL, but not WT LDL or BSA, bound radiolabeled PF4 (Fig. 2A). To examine the consequences of this interaction, we next examined the binding of ox-LDL/PF4 complexes to vascular cells and macrophages. Radiolabeled ox-LDL was incubated with PF4, the complexes...
were isolated by gel filtration, and binding to cells was measured. $^{125}$I-ox-LDL/PF4 complexes exhibited 5-fold more binding to endothelial cells than did $^{125}$I-ox-LDL alone (Fig. 2B). We observed a comparable increase in the binding of PF4-ox-LDL complexes to HVSMC and J774 cells (data not shown).

These data suggest that PF4 and ox-LDL bind to cells as a complex. Based on this finding, our next aim was to define the portion of the complex (ox-LDL or PF4) that mediates cell binding. To address this question, we examined the effect of ox-LDL on the binding of $^{125}$I-PF4 to cells. ox-LDL did not stimulate the binding of PF4 (data not shown). The finding that PF4 stimulated the binding of ox-LDL, whereas ox-LDL did not stimulate the binding of PF4, is consistent with a model wherein PF4 mediates the binding of the complex to the cells and binding of ox-LDL itself does not contribute to this process.

To understand the mechanism by which PF4-ox-LDL complexes bind to cells, we took advantage of the fact that PF4 binds to heparin (48) and to cell surface-associated proteoglycans (49) and that the binding to proteoglycans is inhibited by heparin. Furthermore, heparin does not bind ox-LDL (50). Based on this, we examined the effect of heparin on the binding of ox-LDL to cells. ox-LDL did not stimulate the binding of PF4 (data not shown). The finding that PF4 stimulated the binding of ox-LDL, whereas ox-LDL did not stimulate the binding of PF4, is consistent with a model wherein PF4 mediates the binding of the complex to the cells and binding of ox-LDL itself does not contribute to this process.

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The Role of Cell Surface Proteoglycans in PF4-mediated Binding of ox-LDL—Given the above data, proteoglycans containing glycosaminoglycans that share similarity with heparin and bind PF4 are likely candidates to be the cell surface binding sites for ox-LDL/PF4 complexes. To examine this possibility, we compared the binding of the ox-LDL/PF4 complexes to cells with altered proteoglycan cell surface expression. In these experiments we used two independent approaches. In the first approach, we used a XT CHO cells that are incapable of synthesizing heparan or chondroitin sulfates. These cells bound ox-LDL/PF4 complexes poorly compared with WT CHO cells (Fig. 3). Second, simultaneous digestion of heparan and chondroitin sulfates from the cell surface neutralized the stimulatory effect of PF4 on ox-LDL binding to WT CHO cells by more than 80% (data not shown). Enzymatic cleavage of heparan and chondroitin sulfates also greatly decreased the capacity of HUVEC to bind ox-LDL/PF4 complexes (Fig. 3).

Determinants in PF4 Required to Stimulate ox-LDL Binding—As a third, independent approach toward understanding the requirements for PF4 to promote the binding of ox-LDL to
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cells, we studied the effect of NAP-2, another member of the CXC chemokine family that shares extensive sequence homology with PF4 and demonstrates significant affinity for heparin (51, 52). NAP-2 did not affect the binding of ox-LDL (Fig. 4A).

Two chimeric constructs in which sequences from PF4 had been exchanged with an analogous region of NAP-2 were then used to further define the structural elements in PF4 involved ox-LDL metabolism. These constructs were NPPP, in which the N-terminal eight amino acids before the first of the four conserved cysteine residues in PF4 was replaced with the homologous NAP-2 sequence, and NNNP, in which the complete PF4 sequence from the N terminus to the fourth cysteine residue was replaced with the homologous sequence from NAP-2. Previous studies showed that NPPP binds heparin with the same affinity as PF4, while NNNP binds similarly to both NAP-2 and the lysine to alanine PF4 C-terminal substitutions discussed below (36). NPPP was as effective as PF4 in stimulating ox-LDL binding by HUVEC, whereas NNNP was as ineffective as NAP-2 (Fig. 4A). These studies showed that the N terminus of PF4 is not critically involved in modulating ox-LDL binding.

Consistent with the ability of heparin to block PF4/ox-LDL complexes from binding to cell surfaces (Fig. 2B), the importance of surface heparanoid molecules in complex binding (Fig. 6), and the fact that NAP-2 binds heparin with somewhat lower affinity than does PF4 (37), we next examined the determinants in PF4 required to bind heparin in greater detail and to consider their impact on ox-LDL binding. Each PF4 monomer contains four lysine residues that have been implicated in the binding of heparin and that are located in the C-terminal α-helical domain (20). The results of a previous study (36) show that mutation of any of the four amino acids residues from lysine to alanine reduced heparin-binding affinity of the PF4 tetramer. In the current study, we asked whether these lysine residues were also involved in the capacity of PF4 to stimulate binding of ox-LDL to cells. To do so, we compared the capacity of recombinant PF4 variants with individual Lys to Ala substitutions at positions 62, 65, or 66 to inhibit the binding of 125I-LDL to the cells. (PF4K63A did not express well, and no protein was available to test its function). Whereas PF4K62A and PF4K66A were almost as potent as PF4 both in terms of inhibiting LDL binding and promoting ox-LDL binding to endothelial cells, PF4K65A was less potent than PF4 but more potent than NAP-2 (Fig. 4B). These data suggest that the heparin-binding lysine residues in the C-terminal α-domain of PF4 contribute to the stimulation of ox-LDL binding. However, the data also indicate that although the binding to heparin is important, it appears not to be the only feature as retention of certain Lys residues are more critical than others with PF4K65A retaining nearly normal activity despite having a reduced heparin-binding capacity comparable to NAP-2.

The capacity of NAP-2 and PF4 variants to form complexes with ox-LDL paralleled their capacity to stimulate binding of ox-LDL to cells. Specifically, ox-LDL bound to the chimera NPPP but not to NAP-2 or NNNP (Fig. 5). None of the WT or chimeric chemokines bound to LDL (data not shown), suggesting that the capacity of PF4 to bind to ox-LDL, but not LDL, underlies its divergent effect on the cellular binding of the two lipoproteins. The capacity of each active PF4 variant and the NPPP chimera to stimulate ox-LDL binding was negated in cells genetically lacking or enzymatically stripped of proteoglycans (data not shown), suggesting that each act by the same mechanism as PF4.

Effect of PF4 on ox-LDL Metabolism—Even early atherosclerotic lesions are characterized by macrophage-derived foam cells enriched in cholesterol ester. Therefore, we next examined the effect of PF4 on the esterification of cholesterol from ox-LDL. PF4 increases esterification of ox-LDL cholesterol by
**PF4 Effects on the Vascular Binding of Ox-LDL**

J774 cells almost 10-fold (Fig. 6), while NAP-2 had no effect. Furthermore, Fig. 6 shows that PF4 stimulates the esterification of Ac-LDL cholesterol but not that from native LDL. To study the role of proteoglycans in the PF4-mediated stimulation of ox-LDL esterification, we incubated the cells with heparanase and chondroitinase. Enzymatic cleavage of proteoglycans decreased the capacity of J774 cells to esterify cholesterol from the ox-LDL/PF4 complex by more than 70% (data not shown). These studies show that PF4 binds ox-LDL in vitro, and the resultant complex shows enhanced binding to vascular cells and promote esterification of ox-LDL by J774 cells. To confirm the effect of PF4 on ox-LDL metabolism after incubation of J774 cells with ox-LDL in the presence and absence of PF4, the cell content of cholesterol and cholesterol ester was determined. The presence of PF4 during the incubation increased the cell content of cholesterol from 17 ± 3.3 nmol/mg cell protein at time zero to 104 ± 7.3 after 24 h. In the absence of PF4 the increase of cellular cholesterol was only to 37 ± 4.5 nmol/mg cell protein. Similarly, at the end of the incubation, in the presence of PF4, the cellular content of cholesterol ester from ox-LDL was 9.8 ± 1.4 nmol/mg cell protein compared to 1.3 ± 0.7 in its absence. Similar results were obtained with Ac-LDL (data not shown).

**PF4 and ox-LDL in Atherosclerotic Lesions**—Lastly, to evaluate the relevance of these *in vitro* findings, we examined the localization of PF4 and ox-LDL in human atherosclerotic lesions. Immunohistochemical staining of serial sections of human arteries involved by atherosclerosis demonstrated the presence of both PF4 and ox-LDL in macrophages (Fig. 7). This was true for early lesions (Fig. 7) as well as more advanced lesions (data not shown). That PF4 is present with ox-LDL in early atherosclerotic lesions supports our hypothesis that PF4 may contribute to early lesion formation by increasing the vascular accumulation of ox-LDL.

**DISCUSSION**

The molecular basis of the development of atherosclerosis is complex, multifaceted, and incompletely understood. Platelets may contribute to this process by stimulating lipid uptake by macrophages (10–12). However, the mediators responsible for this activity and the mechanism by which they act have not been defined and merit detailed investigation in an era where chronic administration of inhibitors of platelet activation has become possible.

One potential mediator of platelet involvement in atherosclerosis is PF4, a chemokine that comprises 2–3% of the total protein releasate on a molar basis (21). The biological role of PF4 is unclear, although it clearly binds to heparin and heparanoid side-chains with high affinity. The potential involvement of PF4 in LDL metabolism has been controversial because of two apparently contradictory observations: PF4 inhibits the uptake of LDL by fibroblasts (47), while, on the other hand, platelet activation stimulates macrophage uptake of cholesterol (9–12). Our finding that PF4 inhibits the binding and degradation of LDL by cells but stimulates the binding of ox-LDL offers an explanation for these apparently divergent findings. The effect of PF4 on both forms of the lipoprotein involves its capacity to bind heparin-like molecules. Mutation of the amino acid residues involved in heparin binding decreases its capacity to affect the binding of both subtypes of LDL. Moreover, heparin inhibits the effect of PF4 on both species of LDL.

How does the interaction of PF4 with heparin inhibit the binding of LDL to cells while stimulating ox-LDL binding to the same cells? Our studies provide insight into this aspect of the process by showing that one striking difference is that PF4 binds to ox-LDL but not to LDL. The stimulatory effect of PF4 depends on its ability to bind heparin and ox-LDL simulta-neously. Only WT PF4 and mutants that bind heparin and form complexes with ox-LDL enhance lipoprotein binding to cells. Similarly, chimeric PF4 constructs that bind heparin weakly and/or that do not bind ox-LDL had no stimulatory effect. The mechanism we propose to explain the effect of PF4 on ox-LDL binding to cells is consistent with studies suggesting that several cell types express a low affinity, high capacity pathway for the uptake of oxidized lipoproteins (45).

Taking into consideration our studies and those previously reported, we propose that PF4 released from activated platelets in the vicinity of a perturbed vascular wall stimulates atherosclerosis through the following mechanism: PF4 blocks LDL uptake by the LDL receptor expressed by all of the prevalent cell types in the vascular wall, increasing its retention within the vascular space. Increased residence time in the vasculature permits the lipoprotein to undergo oxidative and non-oxidative modifications. PF4 binds to nascent and preformed ox-LDL. PF4 bridges ox-LDL and cell surface proteoglycans promoting vascular retention. PF4 also enhances the endocytosis and subsequent esterification of the cell-bound ox-LDL in macrophages, accelerating the formation of foam cells. Our data lead us to propose a multistep process by which PF4 accelerates the rate of plasma-derived LDL cholesterol accumulation in the vascular tissue.

PF4 has been implicated to participate in diverse other biologic functions through its strong affinity for heparin-like molecules in addition to those reported in this study. Some of these, such as its chemotactic properties for inflammatory cells (6, 54) and its ability to induce monocytes to differentiate into macrophages (53) may also contribute to the development of atherosclerosis. The fact that PF4 and ox-LDL were found to be co-localized in human atherosclerotic lesions supports the conclusions of our *in vitro* studies. Nevertheless, *in vivo* studies underway in our laboratory in which PF4 concentrations are enhanced or eliminated in transgenic animal models are needed to determine the biological relevance of PF4 for the development of atherosclerosis.

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