Atherosclerosis and Lipoproteins

Balance Between PGD Synthase and PGE Synthase Is a Major Determinant of Atherosclerotic Plaque Instability in Humans

Francesco Cipollone, Maria Fazia, Annalisa Iezzi, Giovanni Ciabattoni, Barbara Pini, Chiara Cuccurullo, Sante Ucchino, Francesco Spigionardo, Mariella De Luca, Cesaria Prontera, Francesco Chiarelli, Franco Cuccurullo, Andrea Mezzetti

Objective—Inducible cyclooxygenase (COX-2) catalyzes the first step in prostanoid biosynthesis and is considered a proinflammatory enzyme. COX-2 and type 1 inducible PGE synthase (mPGES-1) have a role in metalloproteinase (MMP) release leading to plaque rupture. In contrast, lipocalin-type PGD synthase (L-PGDS) has been shown to exert antiinflammatory actions. Thus, in this study we investigated whether a shift from a PGDS-oriented to a PES-oriented profile in arachidonate metabolism leads to inflammatory activation in rupture-prone plaque macrophages.

Methods and Results—Atherosclerotic plaques were obtained from 60 patients who underwent carotid endarterectomy, symptomatic (n=30) and asymptomatic (n=30) according to evidence of recent transient ischemic attack or stroke. Plaques were analyzed for COX-2, mPGES-1, L-PGDS, PPARγ, IkBα, NF-κB, and MMP-9 by immunocytochemistry, Western blot, reverse-transcriptase polymerase chain reaction, enzyme immunoassay, and zymography. Prostaglandin E2 (PGE2) pathway was significantly prevalent in symptomatic plaques, whereas PGD2 pathway was overexpressed in asymptomatic ones, associated with NF-κB inactivation and MMP-9 suppression. In vitro COX-2 inhibition in monocytes was associated with reduced MMP-9 release only when PGD2 pathway overcame PGES pathway.

Conclusions—These results suggest that COX-2 may have proinflammatory and antiinflammatory properties as a function of expression of downstream PGH2 isomerases, and that the switch from L-PGDS to mPGES-1 in plaque macrophages is associated with cerebral ischemic syndromes, possibly through MMP-induced plaque rupture. (Arterioscler Thromb Vasc Biol. 2004;24:1259-1265.)

Key Words: COX-2 ■ PGE synthase ■ PGD synthase ■ metalloproteinase ■ plaque rupture

There is increasing evidence that inflammation plays a central role in the cascade of events that result in plaque erosion and fissuring.1 Lesional macrophages synthesize matrix metalloproteinases (MMPs), proteolytic enzymes capable of degrading plaque constituents.2 In particular, 92 kDa gelatinase (MMP-9), which is specialized in the digestion of collagen fragments, has been critically associated with acute ischemic events in humans.3,4 The MMP-9 promoter contains a clearly defined NF-κB binding site, and NF-κB is therefore one of the transduction pathways that ultimately regulate MMP-9 secretion.5 Furthermore, it has been shown that secretion of MMP-9 by macrophages in human atherosclerotic plaques occurs through a prostaglandin (PG) E2-dependent mechanism.3 PGE2 biosynthesis is influenced by changes in cyclooxygenase (COX) and PGE synthase (PGES) expression.3 Two isoforms of COX and 3 of PGES have been identified, referred to as COX-1 and COX-2 and cytosolic PGES (cPGES) and type 1 and type 2 membrane-bound PGES (mPGES-1 and mPGES-2), respectively. Whereas COX-1 and cPGES are constitutively expressed, COX-2 and mPGES-1 are coregulated in nucleated cells in response to growth factors and cytokines, suggesting that these enzymes are involved in the generation of prostaglandins in inflammatory diseases.3,6 Consistent with the hypothesis of COX-2 and mPGES-1 contributing to the clinical instability of plaques, we recently reported enhanced MMP-9 production by macrophages in symptomatic plaques caused by the enhancement in PGE2 synthesis as a result of the induction of the functionally coupled COX-2/mPGES-1.3

Received January 19, 2004; revision accepted April 26, 2004.
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This study was supported in part by grants from the Italian Ministry of Research and Education (COFIN 2002 and a grant to the Center of Excellence on Aging of the University of Chieti).
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Arterioscler Thromb Vasc Biol is available at http://www.atvbaha.org
DOI: 10.1161/01.ATV.0000133192.39901.be
However, prostaglandins also possess antiinflammatory properties. In fact, circulating PGD synthase (PGDS) has been recently identified as a protective factor in reducing the incidence of restenosis in patients undergoing percutaneous transluminal coronary angioplasty. Furthermore, it has been reported that the PGDS derivative 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2), is a potent inhibitor of NF-κB and IκB and may also exert several antiinflammatory effects through mechanisms involving peroxisome proliferator activated receptor (PPARγ) activation. It is important to acknowledge that staining for 15-deoxy-Δ12,14-PGJ2 has been recently reported in macrophages in human atherosclerotic plaques, together with COX-2 expression.

There are 2 distinct isoforms of PGDS. One is the hematopoietic PGDS (H-PGDS); the other is the lipocalin-type PGDS (L-PGDS), whose expression has been recently reported in human atherosclerotic plaques. Worth attention is the fact that proinflammatory stimuli such as tumor necrosis factor alpha (TNFα) found within atherosclerotic lesions reduced PGD2 synthesis and increased PGE2 release, whereas glucocorticoids may have opposing effects. Thus, the relative abundance of a specific prostanoid is the result of the expression and activity of its specific isomerase, and the predominant expression of mPGES-1 over L-PGDS might lead to increased inflammation and plaque disruption (Figure 1).

Figure 1. Models by which the balance between L-PGDS and mPGES-1 may influence plaque stability during COX-2 overexpression.

Methods

Patients

Between February and November 2001, we studied 60 of 96 consecutive surgical inpatients enlisted to undergo carotid endarterectomy for high-grade (≥70%) internal carotid artery stenosis; these patients were not previously included in other clinical studies. Recruitment was completed when 2 predetermined equal groups of 30 patients according to clinical evidence of plaque instability were achieved. The first group included 30 patients who presented with transient ischemic attack or ischemic stroke (symptomatic patients). The second group included 30 patients who had an asymptomatic carotid stenosis (asymptomatic patients). Percentage of stenosis, procedural methods, concomitant therapy, and risk factors did not differ between the 2 groups (Table I, available online at http://atvb.ahajournals.org). The study was approved by local ethics review committees, and informed written consent was obtained from all patients before each examination.

Reagents

Antibodies anti-human α-actin, anti-CD68, anti-CD3, anti-HLA-DR, anti-CD31 (Dako Corporation, Carpenteria, Calif), anti-MMP-9 (Calbiochem-Novabiochem, San Diego, Calif), anti-COX-1, anti-COX-2, anti-I-PSGDS, anti-mPGES-1, anti-PPARγ (Cayman Chemical, Ann Arbor, Mich), anti-IκBα (Chemicon, Temecula, Calif), and anti-p65α (Roche Biochemical, Mannheim, Germany), were used.

Immunohistochemistry

Plaque sections were prepared and analyzed as previously described. Analysis of immunohistochemistry was performed with a color image analysis system (AlphaEase 5.02; Alpha Innotech Corp).

Western Blot

Western blot analysis was performed as previously described. Bands were quantified by computer-assisted densitometry (Alpha Ease 5.02) and expressed as densitometric unit (DU).

SDS-PAGE Zymography

Zymographic analysis was performed as previously described. Conditioned medium of human fibrosarcoma cell line HT1080 was used as positive control with known gelatinolytic activity.

Reverse-Transcriptase Polymerase Chain Reaction

Reverse-transcriptase polymerase chain reaction for COX-2 was performed as previously described. The GAPDH gene was amplified as internal control. Results were quantified using computer-aided densitometric analysis (Alpha Ease 5.02).

Quantification of NF-κB and PPARγ Activity

Nuclear extracts from plaque specimens were obtained as described by Ohlsson et al. Subsequently, activated p65 was recognized by selective antibody. In addition, activated p50 and PPARγ were measured by specific Trans-AM transcription factor assay kit (Active Motif).

Isolation and Culture of Peripheral Blood Monocytes

The isolation of peripheral monocytes from 5 healthy blood donors was assessed as previously described. Control or stimulated (LPS, 1 μg/mL; IL-1β, 10 ng/mL) monocytes (20×10^6/4 mL of RPMI) were cultured in the presence of aspirin (200 μmol/L) and in the presence or absence of the COX-2 inhibitor NS-398 (1 to 10 μmol/L, for 30'; Cayman), the mPGES-1 inductor TNFα (10 ng/mL for 16 hours, Peprotech Inc, Rocky Hill, NJ), and the inhibitor of mPGES-1 activity buthionine-[S, R]-sulfoximine (50 μmol/L for 18 hours; Sigma). PGH2 (0.42 nM; Cayman), PGE2 (10 μM; Sigma), PGD2 (10 μM; Cayman), or 15d-PGJ2 (5 μmol/L; Cayman) were also added to some of the cultures.
Extraction and Culture of Macrophages From Atherosclerotic Plaques

The extraction of macrophages from 5 symptomatic plaques and 5 asymptomatic plaques was assessed as previously described by de Vries et al. In some experiments, isolated cells were cultured in the presence or absence of the COX-1 inhibitor sulindac sulfide (0.1 to 10 μmol/L; Cayman) and the COX-2 inhibitor NS-398 (0.1 to 10 μmol/L). PGH₂ (0.42 nM; Cayman), PGE₂ (10⁻⁶ M; Sigma), PGD₂, (10⁻⁶ M; Cayman), 15d-PGJ₂ (5 μmol/L; Cayman), the TXA₄ agonist U46619 (10⁻⁶ M; Cayman), the PGL₃ analog carbaprostacyclin (10⁻⁶ M; Cayman), and PGF₁α (200 nM; Cayman) were also added to some of the cultures.

Enzyme Immunoassay, Enzyme-Linked Immunosorbent Assay, and Radioimmunoassay Determinations

Plaque content and in vitro release of PGE₂ (from plaque macrophages), PGD₂ (as its stable methyl oximes), and MMP-9 were measured by enzyme immunoassay (Cayman for PGs, Amersham for MMP-9). In vitro release of PGE₂ from blood monocytes was measured by radioimmunoassay. Plaque content and in vitro release of 15d-PGJ₂ was measured by specific enzyme immunoassay (Assay Designs, Inc, Ann Arbor, Mich). In this assay, the cross-reactivity for a number of related eicosanoid compounds were determined by dissolving the cross-reactant in the assay buffer at reactivities for a number of related eicosanoid compounds were compared by use of the χ² test. The significance of difference in enzyme expression and inflammatory cell infiltration between symptomatic and asymptomatic patients was analyzed by Student t test. The cross-reactivity with PGD₂ was only 4.92% and, more importantly, that with other non-PGDS–dependent prostaglandins was < 0.01%. Finally, cross-reactivity with arachidonic acid was < 0.03%.

Statistical Analysis

For clinical data and histological examination, variables were compared by use of the χ² test. The significance of difference in enzyme expression and inflammatory cell infiltration between symptomatic and asymptomatic patients was analyzed by Student t test. Data are expressed as percentage or mean ± SD. All calculations were performed using the computer program SPSS 10.0.5.

Results

Inflammatory Cell Infiltration

Plaque area occupied by macrophages and T cells was significantly greater (P < 0.0001) in symptomatic than in asymptomatic plaques (Table I). Inflammatory cells in the symptomatic plaques were always characterized by strong expression of HLA-DR antigens, which contrasted markedly with the low expression of HLA-DR in the asymptomatic plaques.

Higher COX-2 Expression in Symptomatic Plaques

COX-2 was more abundant in symptomatic lesions (26 ± 6% versus 7 ± 3%, n = 30, P < 0.0001) (Figure 2). Western blot (Figure 3) and reverse-transcriptase polymerase chain reaction revealed higher COX-2 expression in symptomatic than in asymptomatic plaques (7128 ± 111 versus 2051 ± 353 DU for protein expression, n = 30, P < 0.0001; 8636 ± 251 versus 4633 ± 198 DU for mRNA expression, n = 30, P < 0.0001). In contrast, we did not observe any difference regarding COX-1 expression (4213 ± 85 versus 3987 ± 64 DU, n = 30, P < 0.0001) (Figures 2 and 3).

Figure 2. Stain for COX-1, COX-2, L-PGDS, mPGES-1, and MMP-9 in symptomatic and asymptomatic plaques. Similar regions of the plaque are showed. These results are typical of 30 symptomatic and 30 asymptomatic plaques.

Different Expression of L-PGDS and mPGES-1 in Symptomatic Plaques

Immunohistochemistry revealed strong mPGES-1 immunoreactivity, but only very weak staining for L-PGDS in symptomatic plaques (Figure 2). In contrast, L-PGDS was the predominant isomerase in asymptomatic plaques (Figure 2). This was particularly evident when serial plaque sections were analyzed for L-PGDS and mPGES-1 (Figure 4a). Enzyme staining pattern indicates their localization in macrophages and smooth muscle cells. By quantitative image analysis, levels of mPGES-1 in symptomatic plaques significantly exceeded those of L-PGDS (21 ± 3% versus 5 ± 2%, n = 30, P < 0.0001), whereas the reverse was true in the asymptomatic plaques (4 ± 2% versus 20 ± 4%, n = 30, P < 0.0001). Furthermore, only weak L-PGDS expression was observed in symptomatic plaques by Western blot, whereas a 7-fold higher signal was demonstrated for mPGES-1 (1052 ± 110 versus 7022 ± 322 DU, n = 30, P < 0.0001) (Figure 3). On the contrary, L-PGDS turned out to be the principal isomerase in asymptomatic plaques (5842 ± 128 versus 1223 ± 142 DU, n = 30, P < 0.0001) (Figure 3). However, a direct and accurate comparison of the levels of L-PGDS and mPGES-1 may be limited by variable antibody affinities. Thus, we confirmed results concerning mPGES-1/L-PGDS expression also by enzyme immunoassay measurements of the plaque content of their metabolites. In fact, PGE₂ was
expressing high L-PGDS

Reduced Inflammatory Status in Plaques

Expressing High L-PGDS

PPARγ expression was significantly higher in asymptomatic plaques, with respect to symptomatic plaques, and showed a stable concordance with L-PGDS (6987±125 versus 2142±97 DU for protein expression, n=30, P<0.0001) (Figure 3). In particular, higher PPARγ expression constantly resulted in enhanced PPARγ activity (29±4 versus 21±3 pg/µg of nuclear extracts, n=30; P<0.0001). Similarly, IκBα expression was also significantly higher in asymptomatic plaques (5462±133 versus 1891±109 DU, n=30, P<0.0001) (Figure 3), showed a strong relationship with L-PGDS, and resulted in NF-κB inactivation as reflected by the selective analysis of activated form of p50 (15±4 versus 23±5 pg/µg of nuclear extracts, n=30; P<0.0001) and p65 (1261±142 versus 6411±231 DU, n=30, P<0.0001; Figure 3).

Colocalization of COX-2, L-PGDS, mPGES-1, and MMP-9 in Macrophages of Symptomatic Plaques

In the first experiment, serial plaque sections were incubated with the primary antibodies anti-CD68, anti-COX-2, anti-L-PGDS, anti-mPGES-1, and anti-MMP-9. Within the lesion, staining always accumulated in the CD68+ macrophages in the plaque shoulder. In the second experiment, immunofluorescence double-labeling with confocal microscopy associated the expression of L-PGDS with COX-2 and mPGES-1 in CD68+ cells, both in plaque sections and in plaque-derived macrophages (Figure 4b).

COX-2 but Not COX-1 Is Implicated in the Generation of PGE2 and PGD2

Basal PGE2 and PGD2 release was analyzed in plaque-derived macrophages incubated with NS398 and sulindac sulfide. Results shown in Figure 5a clearly suggest that COX-2 but not COX-1 is involved in PGE2 and PGD2 synthesis.

MMP-9 Production in Monocytes Is Selectively Controlled by PGE2 and PGD2

To determine whether, in addition to PGE2 and PGD2, other COX-derived prostaglandins may influence MMP-9 produc-
tion and plaque stability in the setting of COX-2 overexpression, we examined the effect of PGE₂, PGD₂, 15d-PGJ₂, the TXA₂ agonist U46619, the PGI₂ analog carnaprostacyclin, and PGF₂α on MMP-9 expression in unstimulated macrophages derived from symptomatic plaques. As depicted in Figure 5b, MMP-9 biosynthesis was induced by PGE₂, and inhibited by PGD₂ and 15d-PGJ₂, whereas no effect was observed for the other prostaglandins.

**MMP-9 Production in Monocytes Is a Function of Prevalent PGH₃-Isomerase**

To determine whether a net balance in the isomerase activity in the sense of a mPGES-1-oriented metabolism could lead in turn to MMP-9 production and plaque instability in the setting of COX-2 overexpression, we initially examined the effect of selective mPGES-1 and L-PGDS blockade on NF-κB state and MMP-9 generation in stimulated monocytes in vitro (Figure 5c). According to previous studies, both COX-2 (976 ± 46 versus 4879 ± 123 DU, P < 0.0001) and m-PGES-1 (1098 ± 88 versus 4765 ± 146 DU, P < 0.0001), but not L-PGDS (5889 ± 164 versus 5699 ± 131 DU, P = NS), expression was significantly induced by LPS (Figure 5c).

Worth noting is the fact that LPS caused a strong enhancement in the expression of activated NF-κB (both p65 [879 ± 51 versus 6798 ± 184 DU, P < 0.0001, Figure 5c] and p50 [1021 ± 44 versus 6354 ± 111 DU, P < 0.0001, data not shown] subunits) and MMP-9 (921 ± 34 versus 6574 ± 142 DU, P < 0.0001) (Figure 5c) only in monocytes in which mPGES-1 was induced by LPS and TNFα, but not in those in which mPGES-1 was inhibited by buthionine-[S, R]-sulfoximine. We then examined the effect of selective COX-2 blockade on NF-κB state and MMP-9 generation in the presence of mPGES-1 stimulation or inhibition. Enhanced NF-κB (p65 and p50) and MMP-9 expression by LPS was significantly inhibited by NS-398 in monocytes during mPGES-1 stimulation (1116 ± 77, 1095 ± 63, and 1143 ± 46 DU, respectively; P < 0.0001), and this inhibition was reversed by the addition of PGE₂ (6954 ± 113, 6747 ± 152, and 6543 ± 147 DU, respectively, P < 0.0001) (Figure 5c).

In contrast, COX-2 inhibition in the presence of mPGES-1 inhibition paradoxically increased NF-κB (p65 and p50) activation and MMP-9 generation (1268 ± 135 versus 6975 ± 160, 967 ± 71 versus 6321 ± 122 DU, and 1165 ± 176 versus 6467 ± 128, respectively; P < 0.0001), an effect that was reversed by the addition of PGD₂, or 15d-PGJ₂ (2831 ± 74, 2452 ± 36, and 2598 ± 68 DU, respectively; P < 0.05; Figure 5c). Similar results were also observed when IL-1β, a proinflammatory cytokine usually generated in atherosclerotic lesions, was used as stimulus (data not shown). Thus, NF-κB and MMP-9 activities appear to be critically influenced by the L-PGDS/mPGES-1 balance in inflammatory cells, and COX-2 overexpression per se could be necessary but not sufficient to induce the detrimental biosynthesis of MMP-9 in human atherosclerotic plaques.

**Discussion**

In the present report, we provide evidence for the critical involvement of mPGES-1–oriented arachidonic acid metabolism in MMP-9 overexpression in human symptomatic atherosclerotic plaques. In particular, the present findings are the first, to the best of our knowledge, to: (1) provide evidence for an important antiinflammatory role of L-PGDS in human atherosclerotic lesions, was used as stimulus (data not shown). Thus, NF-κB and MMP-9 activities appear to be critically influenced by the L-PGDS/mPGES-1 balance in inflammatory cells, and COX-2 overexpression per se could be necessary but not sufficient to induce the detrimental biosynthesis of MMP-9 in human atherosclerotic plaques.
flammatory reaction with a PGE$_2$-dominated eicosanoid profile in symptomatic plaques. In fact, in agreement with the difference in COX-2, L-PGDS/mPGES-1, and MMP-9 staining pattern, the histological milieu of the lesions appears different with regard to cellularity, presence of foam cells, and cholesterol clefts, but not in the degree of vessel stenosis. This suggests that asymptomatic and symptomatic lesions are primarily different with regard to the inflammatory burden, and these differences in plaque behavior are likely to derive from differences in the presence of as yet uncharacterized stimuli for the selective expression of L-PGDS or mPGES-1 capable of influencing plaque stability. Notably, this hypothesis is also supported by the evidence of a PGES-prevalent eicosanoid profile in macrophages extracted from symptomatic plaques.

We have previously reported enhanced COX-2 and mPGES-1 expression in symptomatic atherosclerotic lesions. However, this study did not provide any evidence about the real contribution of the antiinflammatory L-PGDS in the pathophysiology of plaque rupture. In fact, COX-2 is only an intermediate enzyme in the metabolic pathway of arachidonic acid, and the COX bioproduct PGH$_2$ is further metabolized by other isomerases to various prostanoids (PGE$_2$, PGD$_2$, PGE$_{2\alpha}$, PGJ$_2$, TXA$_2$). Thus, the relative abundance of a specific prostanoid rather than another is the result of the expression and activity of its specific isomerase, and the concordant inflammatory induction of mPGES-1 with COX-2 in macrophages may lead in turn to a shift in arachidonic acid metabolism from the production of PGD$_2$ to the preferential synthesis of PGE$_2$. The existence of this mechanism has been recently demonstrated in rat peritoneal macrophages in vitro but no evidence exist in human in vivo yet. Our results are consistent with the hypothesis that only the concomitant overexpression of COX-2 and mPGES-1 in the presence of low L-PGDS activity may realize the preferential pathway of arachidonate metabolism leading to increased biosynthesis of PGE$_2$-dependent MMP-9 in the setting of human atherosclerotic plaque. On the contrary, COX-2 overexpression in the presence of a PGD$_2$-dominated eicosanoid profile is associated with enhanced PPAR$_\gamma$ and 1kBo activity, and reduced NF-$\kappa$B activity. Important to take into consideration is the fact that the L-PGDS bioproduct 15d-PGJ$_2$ binds to and activates the nuclear receptor PPAR$_\gamma$. Furthermore, 15d-PGJ$_2$ is also an inhibitor of the activation of NF-$\kappa$B induced by inflammatory cytokines. These data are potentially important, because target genes of NF-$\kappa$B include MMP-9 and COX-2. In support of these evidences, both PGD$_2$ and 15d-PGJ$_2$ were able to completely revert the inflammatory status induced by mPGES-1 in monocytes in vitro in our study. Consequently, the effects of L-PGDS products on PPAR$_\gamma$ and NF-$\kappa$B are consistent with a critical role for this isomerase in plaque stabilization. However, despite the fact that substantial in vitro evidence suggests that 15d-PGJ$_2$ activates PPAR$_\gamma$ and is a potent inhibitor of NF-$\kappa$B, our data seem to suggest that PGD$_2$ rather than 15d-PGJ$_2$ may be responsible for the antiinflammatory action of L-PGDS in human atherosclerotic plaques. In particular, this view is supported by the observation that the stimulatory effect of 15d-PGJ$_2$ on PPAR$_\gamma$ has been observed in vitro at micromolar concentrations, whereas the quantity of 15d-PGJ$_2$ measured in atherosclerotic plaques in our study was significantly smaller and probably insufficient to influence PPAR$_\gamma$ and NF-$\kappa$B activity and, perhaps, to produce any antiinflammatory effect. Thus, we believe that alternative pathways such as direct stimulation of DP receptors by PGD$_2$ and 15d-PGJ$_2$ in target cells should be considered for explaining the anti-inflammatory action of L-PGDS in human atherosclerotic plaques.

The observation that the PG isomerase profile may influence the proinflammatory or antiinflammatory role of COX-2 in atherosclerotic plaques is in agreement with the recent analog demonstration in macrophages in vitro and with the observation that COX-2 overexpression, when associated with minimal PGE$_2$ synthesis and high PGD$_2$ levels, may contribute to the resolution of inflammation. Thus, our results about the antiinflammatory role of COX-2 in human atherosclerotic plaques in the presence of high L-PGDS levels add new data to the recent observations that COX-2 is responsible for increased biosynthesis of the atheroprotective PGJ$_2$ in the setting of atherosclerosis. Furthermore, this study may contribute, at least in part, to explaining the controversial cardiovascular findings in animals and in humans suggesting that COX-2 inhibition may increase the incidence of cardiovascular events, and may have clinical implication for the prolonged use of selective COX-2 inhibitors in patients with coronary artery disease. In fact, COX-2 inhibition in selected patients with an active inflammatory reaction within recently symptomatic (e.g., unstable) atherosclerotic plaques may be beneficial. In contrast, prolonged and widespread use of COX-2 inhibitors in unselected patients with coronary artery disease, most of which have stable, asymptomatic plaques with a low-inflammatory profile, may be detrimental and lead to progressive plaque destabilization.

Finally, L-PGDS may also contribute to the stabilization of COX-2 gene expression in human atherosclerotic plaque during persistent inflammatory stimulation. In fact, 15d-PGJ$_2$ may exert a negative autoregulatory loop on COX-2 gene in macrophages. In contrast, PGE$_2$ produced by mPGES-1 may amplify COX-2 overexpression in the active inflammatory infiltrate of atherosclerotic lesions by an EP4-mediated positive feedback loop, further contributing to progressive plaque destabilization.

In conclusion, this study addresses the missing link between arachidonic acid metabolism and plaque instability by demonstrating the high prevalence of PGJ$_2$-dominated eicosanoid profile in symptomatic atherosclerotic lesions and providing evidence that the shift between L-PGDS and mPGES-1 in activated plaque macrophages is associated with transient ischemic attack and stroke, possibly through MMP-9-induced matrix degradation promoting plaque rupture. These findings are potentially important from a fundamental standpoint, because they demonstrate the critical role of the PGDS/PGES balance in the evolution of atherosclerotic lesions. From a practical standpoint, these findings raise the possibility that drugs able to selectively remodulate arachidonate metabolism in the sense that a PGDS-prevalent path-
way might provide a novel form of therapy for atherosclerotic plaque stabilization.

Acknowledgments
We are grateful to Carlo Patorno for critical reading of this manuscript.

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Arterioscler Thromb Vasc Biol. 2004;24:1259-1265; originally published online May 20, 2004; doi: 10.1161/01.ATV.0000133192.39901.be
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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### Table I. Characteristics of Study Patients.

<table>
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IHD = ischemic heart disease; TIA = Transient Ischemic Attack; NSAID = nonsteroidal anti-inflammatory drug.

*P<0.05; †P<0.0001.