Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis

Miao Wang*, Alicia M. Zukas†, Yiqun Hui*, Emanuela Ricciotti*, Ellen Puré†, and Garret A. FitzGerald**

*Institute for Translational Medicine and Therapeutics, School of Medicine, University of Pennsylvania, 3620 Hamilton Walk, Philadelphia, PA 19104; and †Wistar Institute, 34th and Spruce Streets, Philadelphia, PA 19104

Communicated by Bengt Samuelsson, Karolinska Institutet, Stockholm, Sweden, August 3, 2006 (received for review May 3, 2006)

Prostaglandin (PG) E₂ is formed from PGH₂ by a series of PGE synthase (PGES) enzymes. Microsomal PGES-1^{-/-} (mPGES-1^{-/-}) mice were crossed into low-density lipoprotein receptor knock-out (LDLR^{-/-}) mice to generate mPGES-1^{-/-} LDLR^{-/-}s. Urinary 11 α -hydroxy-9, 15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid (PGE-M) was depressed by mPGES-1 deletion. Vascular mPGES-1 was augmented during atherogenesis in LDLR^{-/-}s. Deletion of mPGES-1 reduced plaque burden in fat-fed LDLR^{-/-}s but did not alter blood pressure. mPGES-1^{-/-} LDLR^{-/-} plaques were enriched with fibrillar collagens relative to LDLR^{-/-}, which also contained small and intermediate-sized collagens. Macrophage foam cells were depleted in mPGES-1^{-/-} LDLR^{-/-} lesions, whereas the total areas rich in vascular smooth muscle cell (VSMC) and matrix were unaltered. mPGES-1 deletion augmented expression of both prostacyclin (PGI₂) and thromboxane (Tx) synthases in endothelial cells, and VSMCs expressing PGI synthase were enriched in mPGES-1^{-/-} LDLR^{-/-} lesions. Stimulation of mPGES-1^{-/-} VSMC and macrophages with bacterial LPS increased PGI₂ and thromboxane A₂ to varied extents. Urinary PGE-M was depressed, whereas urinary 2,3-dinor 6-keto PGF_{1 α} , but not 2,3-dinor-TxB₂, was increased in mPGES-1^{-/-} LDLR^{-/-}s. mPGES-1-derived PGE₂ accelerates atherogenesis in LDLR^{-/-} mice. Disruption of this enzyme retards atherogenesis, without an attendant impact on blood pressure. This may reflect, in part, redirection of accumulated PGH₂ to augment formation of PGI₂. Inhibitors of mPGES-1 may be less likely than those selective for cyclooxygenase 2 to result in cardiovascular complications because of a divergent impact on the biosynthesis of PGI₂.

atherosclerosis | cyclooxygenase | macrophage | vascular smooth muscle cell

Recent placebo-controlled trials have revealed that nonsteroidal antiinflammatory drugs (NSAIDs) selective for inhibition of cyclooxygenase 2 (COX-2) confer a small but absolute risk of myocardial infarction and stroke (1–5). Mechanistically, this is attributable to suppression of COX-2-derived prostacyclin (PGI₂; ref. 6), which acts as a general restraint on endogenous stimuli, including platelet COX-1-derived thromboxane (Tx) A₂, to platelet activation, vascular proliferation and remodeling, hypertension, atherogenesis, and cardiac function (7–13). Precise estimates of the incidence of this risk are not available. However, the relative risk is small and likely to be conditioned by such factors as drug exposure, underlying cardiovascular risk of the patients exposed and concomitant therapies (6, 13). However, the consumption of celecoxib, rofecoxib, and valdecoxib by millions has raised concern that many patients may have suffered cardiovascular adverse events from these drugs.

Inhibitors of COX-2 afford relief from pain and inflammation principally by suppressing prostaglandin (PG) E₂ and PGI₂. Deletion and/or antagonism of receptors for both of these PGs modulate the response to both painful and inflammatory stimuli (14–17). Although deletion of the PGI₂ receptor, the I prostanoid receptor (IP), has revealed the important role that this PG plays in protection of cardiac and vascular function, the role of PGE₂ is more complex. The effects of this PG are transduced by

four E prostanoid receptors (EPs), which mediate contrasting biologies. Two of them, EP2 and EP4, are linked to Gs-mediated activation of adenylate cyclase, whereas the two others, EP1 and EP3, are linked to Gq and/or Gi (18). Deletion of EP2, just like the IP, results in salt-sensitive hypertension (9, 19), whereas EP4 mediates antiinflammatory effects, at least *in vitro* (20, 21). EP1 and EP3 mediate PGE₂-induced vasoconstriction (22, 23), and PGE₂ can either activate platelets by EP3 or, at higher concentrations, inhibit platelet aggregation by the IP (24). A further layer of complexity has been added by the observation that COX-2, EP4, and metalloproteinase (MMP) 9 are all up-regulated in human atherosclerotic plaque *ex vivo* (25), and that COX-2-dependent extracellular matrix-dependent activation of MMP9 is mediated by the EP4 *in vitro* (26).

The product of COX-catalyzed metabolism of arachidonic acid, PGH₂, is metabolized further by specific synthases and isomerases to generate PGs (27). Microsomal (m)-PGE synthase (PGES)-1 (28, 29) is a member of the MAPEG (membrane-associated proteins involved in eicosanoid and glutathione metabolism) superfamily and has previously been suggested as a potential drug target (28). Two other PGES have also been identified, mPGES-2 (30) and cytosolic PGES-1 (31). Recently, mPGES-1 deletion in mice was found to modulate experimentally evoked pain and inflammation to a degree indistinguishable from treatment with traditional COX-nonspecific NSAIDs (32). This raises the possibility that specific inhibitors of mPGES-1 might retain the efficacy of NSAIDs, including those specific for inhibition of COX-2. However, given the contrasting effects of PGE₂ on cardiovascular function, it is unclear whether such a strategy would bypass or diminish the risk evident in trials of NSAIDs specific for inhibition of COX-2. Recently, we have reported that mPGES-1 deletion, in contrast to deletion, disruption, or inhibition of COX-2, does not result in hypertension or a predisposition to thrombosis in normolipidemic mice (13). Here, we consider the impact of mPGES-1 deletion in hyperlipidemic mice. Studies with COX-2 inhibitors have described contrasting effects on atherogenesis in mice, perhaps reflecting variation among the drugs and doses used and both the timing and duration of the intervention (33). Here, we report that

Author contributions: G.A.F. designed research; M.W., A.M.Z., Y.H., and E.R. performed research; E.P. and G.A.F. analyzed data; and M.W., E.P., and G.A.F. wrote the paper.

Conflict of interest statement: G.A.F. receives financial support for investigator-initiated research from Bayer, Merck, and Boehringer Ingelheim, all of which manufacture drugs that target COXs. G.A.F. is a member of the Steering Committee of the Multinational Etoricoxib and Diclofenac Arthritis Long-Term (MEDAL) Study Program. G.A.F. also serves as a consultant for Bayer, Merck, GlaxoSmithKline, Genome Institute of the Novartis Foundation, Boehringer Ingelheim, and NicOx.

Freely available online through the PNAS open access option.

Abbreviations: PG, prostaglandin; PGES, PGE synthase; mPGES, microsomal PGES; PGE-M, 11 α -hydroxy-9, 15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid; LDLR, low-density lipoprotein receptor; VSMC, vascular smooth muscle cell; PGI₂, prostacyclin; PGIS, PGI₂ synthase; Tx, thromboxane; TxS, Tx synthase; PGI-M, 2,3-dinor 6-keto PGF_{1 α} ; Tx-M, 2,3-dinor-TxB₂; NSAID, nonsteroidal antiinflammatory drug; COX, cyclooxygenase; EP, E prostanoid receptor; HFD, high-fat diet; TxA₂, thromboxane A₂; IP, I prostanoid receptor; TxS, TxA₂ synthase.

†To whom correspondence should be addressed. E-mail: garret@spirit.gcrp.upenn.edu.

© 2006 by The National Academy of Sciences of the USA

Fig. 1. mPGES-1 is induced during atherogenesis. (A) mPGES-1 expression was examined by real-time PCR in aorta from mice fed chow diet and after 6-mo HFD. Data shown are the mean $\pm$ SEM from duplicate determinations with $n = 3$ per group. *, $P < 0.05$. (B) mPGES-1 expression in an atherosclerotic lesion. Lesions from LDLR^{-/-} and mPGES-1^{-/-} LDLR^{-/-} mice were stained with blocking peptide-preabsorbed mPGES-1 antibody (Left) and/or the antibody alone (Center and Right). (Scale bar, 100 μ m.)

mPGES-1 is up-regulated in the vasculature during atherogenesis in mice lacking the receptor for low-density lipoprotein (LDLR). Deletion of mPGES-1 results in marked depletion of lesional macrophages and macrophage-derived foam cells, increased expression of vascular smooth muscle cell (VSMC) PGI₂ synthase (PGIS), depression of endogenous PGE₂, and an increase in systemic PGI₂, but not Tx, biosynthesis. These results raise the possibility that drugs that inhibit specifically mPGES-1 may confer cardiovascular benefit, at least in part by redirection of the COX product, PGH₂, to vascular PGIS.

Results

mPGES-1 Deletion Retards Atherogenesis. Neither a gender imbalance nor a genotype-associated developmental morbidity was observed when generating the double-knockout mice (mPGES-1^{-/-} LDLR^{-/-}). Expression of aortic mPGES-1 transcripts increased $\approx$ 2-fold on high-fat diet (HFD; Fig. 1A). Correspondingly, immunostaining of the aortic root revealed mPGES-1 protein expressed in macrophages and macrophage-derived foam cells, particularly in the shoulder region of mPGES-1^{-/-} plaques (Fig. 1B).

Starting at 8 wk of age, the mPGES-1^{-/-} LDLR^{-/-} and control mice (LDLR^{-/-}) of both genders were fed a HFD for 3 or 6 mo. Total plasma cholesterol, triglycerides, and body weight were not different between mPGES-1^{-/-} LDLR^{-/-}s and controls in either gender at any time point. All mice achieved a significant increase in plasma cholesterol during high-fat dietary feeding (Table 1, which is published as supporting information on the PNAS web site).

Deletion of mPGES-1 delayed atherogenesis significantly in both genders in LDLR^{-/-}s (Fig. 2A). The lesion burden decreased in females by a mean 41% and 35% after 3 and 6 mo on HFD and in males by 15% and 50%, respectively. Consistent with the *en face* data, crosssectional analysis of aortic root samples revealed a significant decrease (34%) in the total lesion area in the aortic root from female mice fed HFD for 6 mo (Fig. 2B). mPGES-1 deletion did not affect blood pressure in either

females [116.7 ± 5.2 mmHg in LDLR^{-/-} vs. 113.3 ± 3.6 mmHg in mPGES-1^{-/-} LDLR^{-/-} (1 mmHg = 133 Pa)] or males (112.8 ± 3.3 mmHg in LDLR^{-/-} vs. 113.4 ± 2.7 mmHg in mPGES-1^{-/-} LDLR^{-/-}), as measured after 6 mo on the HFD ($P > 0.8$, $n = 10$ –15 per group).

Lesional Morphology Consequent to mPGES-1 Deletion in LDLR^{-/-} Mice. Macrophages, VSMCs, and collagen were immunostained in plaques of comparable size obtained at the aortic roots (Fig. 3). The VSMC content was variable in both groups, with no consistent difference observed between the groups in VSMC abundance (Fig. 3A Left and B) or the percentage of lesion matrix rich (i.e., collagen) area. However, the birefringence of sirius red staining under polarized light indicates that the collagen that accumulates in the lesions of mPGES-1^{-/-} LDLR^{-/-} mice is in the form of mid-sized to large fibers (yellow to white reflection) typical of type I collagen compared with small fibers (red reflection) evident in the LDLR^{-/-} (Fig. 3A Right). Strikingly, macrophages, as stained by CD11b (Fig. 3A Center), were depleted in lesions from the mPGES-1^{-/-} LDLR^{-/-}s compared with littermate controls. Indeed, macrophage foam cells, as stained by CD11c antibody, occupied $\approx$ 60% of the total plaque area in LDLR^{-/-}s, contrasting $\approx$ 40% in the mPGES-1^{-/-} LDLR^{-/-}s (Fig. 3C).

Both PGIS and thromboxane A₂ (TxA₂) synthase (TxS) were detectable in LDLR^{-/-}s (Fig. 5, which is published as supporting information on the PNAS web site). TxS was particularly evident in macrophage foam cells and PGIS in VSMC. Lesions obtained from mPGES-1^{-/-} LDLR^{-/-}s had less TxS, reflecting macrophage depletion; they also exhibited less necrosis of the lesional core. Although structural integrity of the endothelium was retained over the disrupted sections of vasculature, and the endothelium was morphologically indistinguishable between the two groups, both PGIS and TxS were much more abundantly expressed in endothelium in the mPGES-1^{-/-} LDLR^{-/-}s, suggesting a functional distinction. Endothelial localization of the two synthases was supported by apparent colocalization with lesional CD31 (platelet endothelial cell adhesion molecule, PECAM). PGIS was relatively more abundant in mPGES-1^{-/-} LDLR^{-/-} lesions, probably due to macrophage depletion and the relative abundance of medial VSMCs, which are rich in the enzyme. Strikingly, VSMCs were radially orientated in mPGES-1^{-/-} LDLR^{-/-} lesions, perhaps reflecting the relative deficiency, compared with LDLR^{-/-}, of migratory cues from necrotic or foam cell regions.

Differential Impact of mPGES-1 Deletion on Prostanoid Generation in Macrophages and VSMCs. mPGES-1 was the primary source of PGE₂ formation under basal conditions and after LPS stimulation of either macrophages or VSMCs (Fig. 6, which is published as supporting information on the PNAS web site). VSMCs were a more abundant source of 6-keto PGF_{1 α} (the hydrolysis product of PGI₂) than macrophages under basal conditions. Also, the relative abundance of prostanoid formation in macrophages (PGE₂ > TxB₂ > 6-keto PGF_{1 α}) differed from that in VSMC (6-keto PGF_{1 α} > PGE₂ > TxB₂).

Deletion of mPGES-1 depressed PGE₂ and augmented LPS-stimulated 6-keto PGF_{1 α} and TxB₂ in both cell types, although the principal redirection product in VSMC was PGI₂, whereas TxA₂ predominated in the macrophages. Indeed, under basal conditions, the only evidence of substrate shift detectable was a significant increase in 6-keto PGF_{1 α} production in VSMC.

mPGES-1 Deletion Decreases Systemic Biosynthesis of PGE₂ and Augments PGI₂. Systemic production of PGE₂, TxA₂, and PGI₂ was determined by measuring their major urinary metabolites, 11 α -hydroxy-9, 15-dioxo-2,3,4,5-tetranor-prostanoic acid (PGE-M), 2,3-dinor-TxB₂ (Tx-M), and 2,3-dinor 6-keto PGF_{1 α}

Fig. 2. mPGES-1 deletion retards atherogenesis in LDLR^{-/-} mice. (A) The extent of atherosclerosis, represented by the ratio of lesion area to total aortic area, was quantified by *en face* analysis of aortae from mice treated with a HFD for 3 or 6 mo (Upper). Representative *en face* graphs are shown (Lower), with each vertically matched to the corresponding data set in Upper. (B) Box-and-whiskers graph revealing the crosssectional analysis of aortic root samples from female mice after 6 mo on HFD, measuring total lesion area across the aortic root as detailed in Materials and Methods (Upper). A representative crosssection from each group is shown (Lower), vertically aligned with the corresponding data in Upper. (Scale bar, 500 μ m.) *, $P < 0.05$; **, $P < 0.01$; $n = 10$ –15 per group.

(PGI-M), respectively. mPGES-1 deletion significantly decreased PGE-M in the mPGES-1^{-/-} LDLR^{-/-}s in each gender before HFD treatment (Fig. 7, which is published as supporting informa-

tion on the PNAS web site). Urinary Tx-M and PGI-M both increased during atherogenesis in LDLR^{-/-}s (Fig. 4). Urinary PGI-M increased further in the mPGES-1^{-/-} LDLR^{-/-}s, which was evident at all time points in the study, whereas excretion of Tx-M was unaltered. The significant depression of urinary PGE-M in mPGES-1-deleted mice was sustained on the HFD at 3 (12.33 ± 1.84 vs. 6.08 ± 1.05 ng/mg creatinine, $P < 0.01$ $n = 14$ and 16, respectively) and 6 mo (8.74 ± 0.99 vs. 5.16 ± 0.89 ng/mg creatinine, $P < 0.05$ $n = 11$ per group).

Discussion

Development of drugs that inhibit specifically mPGES-1 as alternatives to NSAIDs is configured on several assumptions. These include the premises that (i) mPGES-1 is a major source of the PGE₂ formed *in vivo*, and that other PGES enzymes do not substitute for it when it is inhibited; (ii) an mPGES-1 inhibitor retains substantially the clinical efficacy of NSAIDs, despite the recognition that PGs other than PGE₂, particularly PGI₂, may contribute to pain and inflammation; (iii) the cardiovascular hazard attributable to specific inhibitors of COX-2 results from inhibition of PGI₂ rather than PGE₂; and (iv) the gastrointestinal consequences of mPGES-1 inhibition do not exceed those of NSAIDs, particularly those selective for COX-2.

The present study was designed to test some of these assumptions. We have previously reported that deletion of mPGES-1 depresses substantially endogenous PGE₂ biosynthesis, as reflected by urinary PGE-M, in normolipidemic mice (13). Here, it is apparent that this observation extends to hyperlipidemic mice, and this inhibition is sustained as they develop atherosclerosis on a HFD. Thus, other PGES do not appear to substitute

Fig. 3. Effects of mPGES-1 deletion on lesional morphology in LDLR^{-/-} mice. Smooth muscle cells and macrophages were stained by antibodies against α -smooth muscle actin (α -SMA; *A Left*) and CD11b (*A Center*), respectively. Macrophages were depleted in the mPGES-1^{-/-} LDLR^{-/-}s. Collagen content was stained by sirius red (*A Right*). Note the fibrillar collagen content of the mPGES-1^{-/-} LDLR^{-/-} neointima. All stainings in *A* were performed on one lesion of each genotype. Another set of α -SMA staining is shown in *B*. [Scale bar, 200 μ m (*A*) and 100 μ m (*B*).] Quantitative analysis (*C*) of macrophage-foam cells reveals a significant decrease in the ratio of foam cell area to lesion area. *, $P = 0.02$.

Fig. 4. Biosynthesis of PGI₂ and TxA₂ during atherogenesis. Systemic production of PGI₂ and TxA₂ was examined by measuring their urinary metabolites, PGI-M and Tx-M. Both increased during atherogenesis in the LDLR^{-/-} mice and substrate redirection to PGI₂ (A), but not TxA₂ (B), is observed in response to mPGES-1 deletion. This is sustained during the study period in hyperlipidemic mice. Data shown are from females. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $n = 11$ –16 per group.

for the suppression of PGE₂ formation consequent to mPGES-1 deletion.

We have previously reported that mPGES-1 deletion does not result in an elevation of blood pressure or enhance the response to thrombogenic stimuli in normolipidemic mice in which genetic manipulation or inhibition of COX-2 adversely influences both variables (13). Here again, we fail to observe a rise in blood pressure, this time when mPGES-1^{-/-} mice are crossed into those lacking the LDLR. Indeed, deficiency in mPGES-1 does not adversely affect blood pressure during prolonged HFD feeding.

The impact of COX-2 deficiency on atherogenesis is complex. Investigators have reported that the development of plaque burden is accelerated, retarded, or uninfluenced by COX-2 inhibitors. These contrasting effects may reflect the contrasting biologies of the products of COX-2 formed by cells that predominate variably at different stages in disease evolution (33). Here, we observe that atherogenesis results in increased vascular expression of mPGES-1 in LDLR^{-/-} mice. Deletion of mPGES-1 retards atherogenesis strikingly on an LDLR^{-/-} background.

Lesion morphology is markedly altered in mPGES-1^{-/-} LDLR^{-/-}s. Macrophages, themselves rich in COX-2, TxS, and mPGES-1, are markedly depleted. The VSMC content of the lesions, by contrast, is apparently unaltered. Interestingly, we observed changes in the type of collagen that accumulates in the atherosclerotic lesions, based on birefringence analysis of sirius-

stained sections. Specifically, whereas lesions in LDLR^{-/-}s have significant levels of smaller fibers, the mPGES-1^{-/-} LDLR^{-/-} lesions were enriched in fibrillar collagens (presumably type I, III, and or V), the predominant collagens produced by VSMC. These changes and the depletion of lesional macrophages are two of three morphological hallmarks of plaque stabilization (33), evident when mPGES-1 deletion occurs in hyperlipidemic mice. The third, enhanced formation of a fibrous cap, was not observed. However, there was no suggestion of plaque destabilization (25) in the mPGES-1^{-/-} LDLR^{-/-}s. Expression of both PGIS and TxS is increased in endothelial cells, and the increase in the ratio of VSMCs relative to foam cells is associated with an increase in the proportion of neointimal cells expressing PGIS consequent to deletion of mPGES-1. Increased endothelial expression of both PGIS and TxS suggests removal of an mPGES-1-derived regulatory restraint on these enzymes in the mPGES-1^{-/-} LDLR^{-/-}s.

Trebino *et al.* (34) have previously reported that synthesis of both TxA₂ and PGI₂ is increased in LPS-stimulated macrophages obtained from mPGES-1^{-/-} mice *ex vivo*, which they attributed to redirection of the accumulated PGH₂ substrate to PGIS and TxS. Here, we replicate their observation in macrophages. Synthesis of both PGI₂ and TxA₂ is increased under LPS-stimulated conditions in mPGES-1-depleted macrophages. However, whereas this is also true of LPS-stimulated VSMCs, PGI₂ is the more abundant product, and only PGI₂ formation is increased in mPGES-1-depleted VSMC under basal conditions. Thus, the predominant product formed consequent to PGH₂ redirection due to mPGES-1 deletion or inhibition is likely to vary by cell type and pathophysiological condition (Fig. 8, which is published as supporting information on the PNAS web site).

Analysis of urinary metabolites represents a time-integrated noninvasive approach to the study of PG biosynthesis *in vivo* (35). Previously, we have shown that mPGES-1 deletion augments PGI₂, but not TxA₂, metabolite excretion in normolipidemic mice in which PGE₂ biosynthesis is suppressed substantially. Here, we show that in the mPGES-1^{-/-} LDLR^{-/-}s, systemic biosynthesis of PGI₂, but not TxA₂, is augmented, and that this increase is sustained during atherogenesis. This may reflect the increased vascular expression of PGIS, together with depletion of lesional macrophages, a major source of TxA₂ (36). Despite the marked gender difference in biosynthesis of PGE₂, apparent both in normolipidemic and here in hyperlipidemic mice, the qualitative shift to an increase in PGI₂ biosynthesis is apparent in both males and females. It is possible that initial manipulation of mPGES-1 when atherosclerosis is advanced and lesions are rich in macrophages might augment Tx biosynthesis. However, inducible deletion of the enzyme or timed intervention with a specific inhibitor would be necessary to address this hypothesis.

Previous studies have indicated that PGI₂ is an important restraint on initiation and early development of atherogenesis in both male (10) and female (11) hyperlipidemic mice. This appears to reflect, at least in part, the induction of antioxidant enzymes, such as hemeoxygenase-1 (11, 37), which serve to counter free radical induced vascular injury consequent to platelet (10, 11) and neutrophil (10) activation. Furthermore, PGIS is uniquely susceptible to free radical-based inactivation (38), such as might pertain during atherogenesis. Although biosynthesis of PGI₂ is augmented in patients with severe atherosclerosis (39), this reflects accelerated platelet and neutrophil-vessel wall interactions and stimulated production in response to physical and chemical stimuli. The capacity of atherosclerotic vascular tissue to produce PGI₂, by contrast, is reduced compared with healthy vasculature (39). Biosynthesis of PGI₂ is augmented by mPGES-1 deletion by ~50% in both normolipidemic and hyperlipidemic mice. Although the functional relevance of this observation is unknown, haploinsuffi-

PNAS | September 26, 2006 | vol. 103 | no. 39 | 14511

compared, the two-tailed Mann–Whitney *t* test was used. Differences were considered statistically significant at *P* < 0.05.

Technical support was kindly provided by Helen Zou and Ping Liu. We gratefully acknowledge the contributions of Karine Egan, Julien

Ferrari, and John Lawson. mPGES-1^{-/-} mice were generously supplied by Dr. L. Audoly (Pfizer, New York, NY). This work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Grants HL 70128, HL 62250, and 2-P01-HL-06225). G.A.F. holds the Elmer Bobst Chair of Pharmacology.

- Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, *et al.* (2000) *N Engl J Med* 343:1520–1528, 2 p following 1528.
- Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanus A, *et al.* (2005) *N Engl J Med* 352:1092–1102.
- Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnoli M (2005) *N Engl J Med* 352:1071–1080.
- Furberg CD, Psaty BM, FitzGerald GA (2005) *Circulation* 111:249.
- Anonymous (2005) *Public Citizen*, www.citizen.org/publications/release.cfm?ID=7358.
- Grosser T, Fries S, FitzGerald GA (2006) *J Clin Invest* 116:4–15.
- Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA, FitzGerald GA (2002) *Science* 296:539–541.
- Rudic RD, Brinster D, Cheng Y, Fries S, Song WL, Austin S, Coffman TM, FitzGerald GA (2005) *Circ Res* 96:1240–1247.
- Francois H, Athirakul K, Howell D, Dash R, Mao L, Kim HS, Rockman HA, FitzGerald GA, Koller BH, Coffman TM (2005) *Cell Metab* 2:201–207.
- Kobayashi T, Tahara Y, Matsumoto M, Iguchi M, Sano H, Murayama T, Arai H, Oida H, Yurugi-Kobayashi T, Yamashita JK, *et al.* (2004) *J Clin Invest* 114:784–794.
- Egan KM, Lawson JA, Fries S, Koller B, Rader DJ, Smyth EM, FitzGerald GA (2004) *Science* 306:1954–1957.
- Hennan JK, Huang J, Barrett TD, Driscoll EM, Willens DE, Park AM, Crofford LJ, Lucchesi BR (2001) *Circulation* 104:820–825.
- Cheng Y, Wang M, Yu Y, Lawson J, Funk CD, FitzGerald GA (2006) *J Clin Invest* 116:1391–1399.
- Takasaki I, Nojima H, Shiraki K, Sugimoto Y, Ichikawa A, Ushikubi F, Narumiya S, Kuraishi Y (2005) *Neuropharmacology* 49:283–292.
- Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M (2005) *Mol Pain* 1:3.
- Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, Ichikawa A, Aze Y, Tanaka T, Yoshida N, *et al.* (1997) *Nature* 388:678–682.
- Honda T, Segi-Nishida E, Miyachi Y, Narumiya S (2006) *J Exp Med* 203:325–335.
- Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, *et al.* (1998) *Nature* 395:281–284.
- Kennedy CR, Zhang Y, Brandon S, Guan Y, Coffee K, Funk CD, Magnuson MA, Oates JA, Breyer MD, Breyer RM (1999) *Nat Med* 5:217–220.
- Takayama K, Garcia-Cardena G, Sukhova GK, Comander J, Gimbrone MA, Jr, & Libby P (2002) *J Biol Chem* 277:44147–44154.
- Takayama K, Sukhova GK, Chin MT, Libby P (2006) *Circ Res* 98:499–504.
- Norel X, de Montpreville V, Brink C (2004) *Prostaglandins Other Lipid Mediat* 74:101–112.
- Jadhav V, Jabre A, Lin SZ, Lee TJ (2004) *J Cereb Blood Flow Metab* 24:1305–1316.
- Fabre JE, Nguyen M, Athirakul K, Coggins K, McNeish JD, Austin S, Parise LK, FitzGerald GA, Coffman TM, Koller BH (2001) *J Clin Invest* 107:603–610.
- Cipollone F, Fazia ML, Iezzi A, Cuccurullo C, De Caesere D, Uccino S, Spigonardo F, Marchetti A, Buttita F, Paloscia L, *et al.* (2005) *Arterioscler Thromb Vasc Biol* 25:1925–1931.
- Pavlovic S, Du B, Sakamoto K, Khan KM, Natarajan C, Breyer RM, Danenberg AJ, Falcone DJ (2006) *J Biol Chem* 281:3321–3328.
- Funk CD (2001) *Science* 294:1871–1875.
- Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B (1999) *Proc Natl Acad Sci USA* 96:7220–7225.
- Thoren S, Weinander R, Saha S, Jegerschold C, Pettersson PL, Samuelsson B, Hebert H, Hamberg M, Morgenstern R, Jakobsson PJ (2003) *J Biol Chem* 278:22199–22209.
- Murakami M, Kudo I (2006) *Curr Pharm Des* 12:943–954.
- Pini B, Grosser T, Lawson JA, Price TS, Pack MA, FitzGerald GA (2005) *Arterioscler Thromb Vasc Biol* 25:315–320.
- Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, Saha S, Roach ML, *et al.* (2003) *Proc Natl Acad Sci USA* 100:9044–9049.
- Egan KM, Wang M, Fries S, Lucitt MB, Zukas AM, Pure E, Lawson JA, FitzGerald GA (2005) *Circulation* 111:334–342.
- Trebino CE, Eskra JD, Wachtmann TS, Perez JR, Carty TJ, Audoly LP (2005) *J Biol Chem* 280:16579–16585.
- FitzGerald GA, Pedersen AK, Patrono C (1983) *Circulation* 67:1174–1177.
- Burleigh ME, Babaev VR, Oates JA, Harris RC, Gautam S, Riendeau D, Marnett LJ, Morrow JD, Fazio S, Linton MF (2002) *Circulation* 105:1816–1823.
- Meyer-Kirchath J, Debey S, Glandorff C, Kirchath L, Schror K (2004) *Biochem Pharmacol* 67:757–765.
- Zou MH, Shi C, Cohen RA (2002) *Diabetes* 51:198–203.
- FitzGerald GA, Smith B, Pedersen AK, Brash AR (1984) *N Engl J Med* 310:1065–1068.
- Tangirala RK, Rubin EM, Palinski W (1995) *J Lipid Res* 36:2320–2328.