

# Modulation of Human Monocyte Activities by Tranilast, SB 252218, a Compound Demonstrating Efficacy in Restenosis

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## ABSTRACT

Tranilast (SB 252218) is a compound initially identified as an anti-atopic agent. Recently the compound has demonstrated clear beneficial effects in animal models of restenosis. Here we confirm tranilast has broad and profound effects on human monocytes, which could contribute to the vascular antifibrotic activity. Tranilast exhibited significant immunomodulatory activity inhibiting endotoxin-induced prostaglandin E<sub>2</sub> (PGE<sub>2</sub>; IC<sub>50</sub> = ~1–20 μM), thromboxane B<sub>2</sub> (IC<sub>50</sub> = ~10–50 μM), transforming growth factor-β1 (TGF-β1; IC<sub>50</sub> = ~100–200 μM), and interleukin-8 (IC<sub>50</sub> = ~100 μM) formation, but had no effect on tumor necrosis factor-α. Interleukin-12 and -18-induced interferon-γ formation by monocytes was also attenuated by tranilast. A23187-induced monocyte leukotriene C<sub>4</sub> or PGE<sub>2</sub> formation was inhibited by tranilast at IC<sub>50</sub> values of 10–40 μM and 2–20 μM, respectively, incubated with or without exoge-

nous arachidonic acid. Interestingly, tranilast (up to 1000 μM) had no direct effects on cyclooxygenase I or II activity, nor did it have significant effects on human type IIA 14 kDa or type IV 85 kDa phospholipase A<sub>2</sub> activity. Furthermore, tranilast had no effect on endotoxin-induced cyclooxygenase II protein expression, suggesting tranilast modulates eicosanoid production and release by an as yet unidentified mechanism. Alternatively, the expression of TGF-β1 was inhibited by tranilast but found to be due in part to inhibition of PGE<sub>2</sub> because exogenous PGE<sub>2</sub> could abrogate tranilast-mediated inhibition of TGF-β1. Taken together, although a reported direct inhibitor of fibroblast proliferation, we show tranilast also attenuates the proinflammatory activity of human monocytes, adding to its potential efficacy as a therapeutic agent in restenosis.

Since its introduction in 1978 (Gruntzig, 1978), percutaneous transluminal coronary angioplasty (PTCA) has been universally accepted as the interventional revascularization procedure of choice to relieve symptoms associated with atherosclerotic vascular disease. Nearly 500,000 PTCA were done in the United States in 1996 (American Heart Association, 1999, Heart and Stroke A-Z Guide, <http://www.amhrt.org>). Ninety-five percent of patients experience immediate revascularization. However, 25 to 30% of patients experience significant restenosis within 6 months, requiring additional intervention, including repeat PTCA and/or by-pass surgery (American Heart Association, 1999, Heart and Stroke A-Z Guide, <http://www.amhrt.org>). Proliferation of vascular smooth muscle cells, deposition of extracellular matrix, and infiltration of inflammatory cells with release of inflammatory mediators are implicated both in the pathogenesis of primary atherosclerosis and the vascular restenosis seen af-

ter interventional procedures (Ross, 1999). Thus, regulators of vascular smooth muscle cell proliferation, matrix metabolism, and inflammation are critical potential targets for development of drugs to prevent restenosis.

Tranilast, *N*-(3,4-dimethoxycinnamoyl) anthranilic acid (SB 252218), is currently used in Japan as an antiasthma drug (Azuma et al., 1976). Initially identified as an inhibitor of mast cell degranulation (Komatsu et al., 1988a), this compound was later found to prevent keloid scarring, presumably through its antiproliferative effects on fibroblasts (Suzawa et al., 1992a). Recently, tranilast was shown, in clinical trials, to prevent restenosis after PTCA (Tamai et al., 1999). The mechanisms by which tranilast inhibits restenosis are likely due to the cumulative effects of its antiproliferative and its immunomodulatory action. It is unlikely, however, that mast cells play an important part because mast cell-deficient mice readily undergo tranilast-inhibitable fibrosis after injury (Mori et al., 1991). Other inflammatory cells,

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**ABBREVIATIONS:** PTCA, percutaneous transluminal coronary angioplasty; PG, prostaglandin; LT, leukotriene; TGF, transforming growth factor; IL, interleukin; COX, cyclooxygenase; PBMC, peripheral blood mononuclear cell; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IFN, interferon; DMSO, dimethyl sulfoxide; AA, arachidonic acid; ELISA, enzyme-linked immunosorbent assay; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; rh, recombinant human; TLC, thin-layer chromatography; PAGE, polyacrylamide gel electrophoresis; TX, thromboxane.

lymphocytes, tissue macrophages, and circulating blood monocytes are implicated in fibrotic events, including restenosis (Serrano et al., 1997). Indeed, tranilast has been shown to inhibit prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), leukotriene C<sub>4</sub> (LTC<sub>4</sub>), transforming growth factor-β1 (TGF-β1), and interleukin-1β (IL-1β) release from mast cells and macrophages (Komatsu et al., 1988b; Suzawa et al., 1992b) and can reduce expression of cell surface markers such as major histocompatibility complex class II and IL-2 receptors (Kawano and Noma, 1993; Matsumura et al., 1999).

The exact mechanism for tranilast's immunosuppressive activities remains unknown. It has been shown to block calcium entry into mast cells (Komatsu et al., 1988a). But recent data show it has no effect on the release of calcium from intracellular stores (Nie et al., 1996, 1997). Early studies suggest a lack of direct effect on the activities of eicosanoid-synthesizing enzymes such as the 5-lipoxygenase or cyclooxygenase (COX) (Komatsu et al., 1988b). It is hypothesized tranilast may also effect additional signal transduction mechanisms such as cAMP levels and induction of phosphatidylinositol turnover (Komatsu et al., 1988a).

Herein we confirm and further demonstrate tranilast has broad and profound effects on monocyte-enriched human peripheral blood mononuclear cell (PBMC) function. More in depth studies to elucidate mechanism of action indicate tranilast directly inhibits A23187 and endotoxin (lipopolysaccharide, LPS)-induced monocyte-enriched human PBMC PGE<sub>2</sub> and LTC<sub>4</sub> formation, as well as LPS-induced TGF-β1 expression. We show for the first time that tranilast inhibits LPS-induced IL-8 formation but not tumor necrosis factor-α (TNF-α) release, and modulates PBMC interferon-γ (IFN-γ) formation after treatment with IL-12 and IL-18. Additionally, we show inhibition of prostanoid synthesis is not due to down-regulation of the inducible COX-II enzyme, nor is it due to a decrease in activity, suggesting an as yet unidentified mode of action in eicosanoid modulation. We demonstrate for the first time that tranilast can modify TGF-β1 production by attenuating both protein and mRNA levels and finally, that PGE<sub>2</sub> is able to abrogate tranilast inhibition of TGF-β1, indicating that TGF-β1 inhibition may be secondary to PGE<sub>2</sub>-reducing activities. These direct effects on mediator release suggest that tranilast efficacy in restenosis may be due to a combination of its immunomodulatory activities as well as its effects on fibroblast and smooth muscle cell proliferation.

## Materials and Methods

**Human Monocyte-Stimulated Eicosanoid and Cytokine Production.** Monocytes were isolated from heparinized whole blood by double gradient centrifugation as previously described (Marshall et al., 1997). Isolated monocyte-enriched PBMCs were then adhered to 24-well culture plates at  $2 \times 10^6$  cells/ml in RPMI-1640 10% fetal bovine serum (Hyclone, Logan, UT) for 2 h to further enrich the monocyte population. The media were then removed, cells washed once with RPMI-1640, and 1 ml of RPMI-1640 10% fetal bovine serum was added to the wells. Test compounds were added to the wells with a final vehicle concentration of 0.5% DMSO. Monocytes were activated by the addition of 200 ng/ml endotoxin (LPS; *Escherichia coli* serotype 026:B6) (Sigma, St. Louis, MO) or A23187 (1 μM) and incubated for 24 h or 7 min, respectively. Some assays were performed with or without the addition of exogenous arachidonic acid (AA, 20 μM; Sigma). For specific activation by cytokines, cells were incubated with 5 to 10 nM IL-12 (Pharmingen, San Diego, CA)

and/or IL-18 for 24 h. Recombinant human IL-18 was expressed and purified in the Department of Gene Expression Sciences, SmithKline Beecham Pharmaceuticals. Cell-free supernatants were analyzed by ELISA for TNF-α (developed at SmithKline Beecham), PGE<sub>2</sub>, and LTC<sub>4</sub> (Cayman Chemical, Ann Arbor, MI), TGF-β1 (no cross-reactivity with TGF-β2 or 3; Genzyme Corp., Cambridge, MA) and IL-8 and IFN-γ (Biosource International, Camarillo, CA). Viability of the cells was determined by Trypan blue exclusion.

**Assessment of Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and Cyclooxygenase I and II Activities.** PLA<sub>2</sub> activity of isolated recombinant enzymes was measured by the acylhydrolysis of [<sup>3</sup>H]AA *E. coli* as previously described (Marshall et al., 1991). Recombinant human (rh-) type IIA 14-kDa PLA<sub>2</sub> or rh-85-kDa PLA<sub>2</sub> were added to no more than 0.5 to 5 ng of protein per assay. Vehicle (DMSO) or drug solubilized in DMSO was added to no greater than 10% of the total assay volume. Tranilast or vehicle was incubated with the enzyme for 10 min at 27°C before substrate addition unless otherwise stated. Results are calculated as percentage of free fatty acid hydrolyzed [sample dpms generated minus background (nonspecific hydrolysis) dpms divided by total dpms added  $\times$  100].

**In Vitro Assays of Cyclooxygenase Activity.** Microsomal membrane preparations of COS-1 cells transfected with either the expression plasmid pOSML-PGHS-1 or pOSML-PGHS-2, which contain the cDNAs for the human COX-I and COX-II enzymes, respectively, were used as the enzyme source for measurements of inhibition of cyclooxygenase activity. Oxygen electrode assays were conducted as previously described (Laneuville et al., 1994). To measure instantaneous inhibition, 100 μg of total microsomal protein was added to cuvettes containing 10 μM arachidonic acid, 28 μg/ml hemoglobin, 1 mM phenol, and the indicated concentration of tranilast or the nonspecific COX inhibitor flurbiprofen, in 3 ml of 0.1 M Tris-HCl, pH 8.0. For whole-cell assays, COS-1 cells transfected with the expression plasmids were harvested after 40 h and resuspended in Dulbecco's modified Eagle's medium at a concentration of  $2 \times 10^7$  cells/ml. Alternatively, cell suspensions (250 μl) were preincubated with flurbiprofen, tranilast, or vehicle for 5 min at 37°C. [<sup>14</sup>C]arachidonic acid (53 mCi/mM) (NEN, Boston, MA) was added and the samples were incubated at 37°C for 10 min. Cells were then removed by centrifugation at 1000g, the supernatants were extracted, and the products were separated by TLC as previously described (Laneuville et al., 1994). After overnight exposure of the TLC plates to a storage phosphor screen, relative production of <sup>14</sup>C-prostaglandin products was determined using a Molecular Dynamics PhosphorImager.

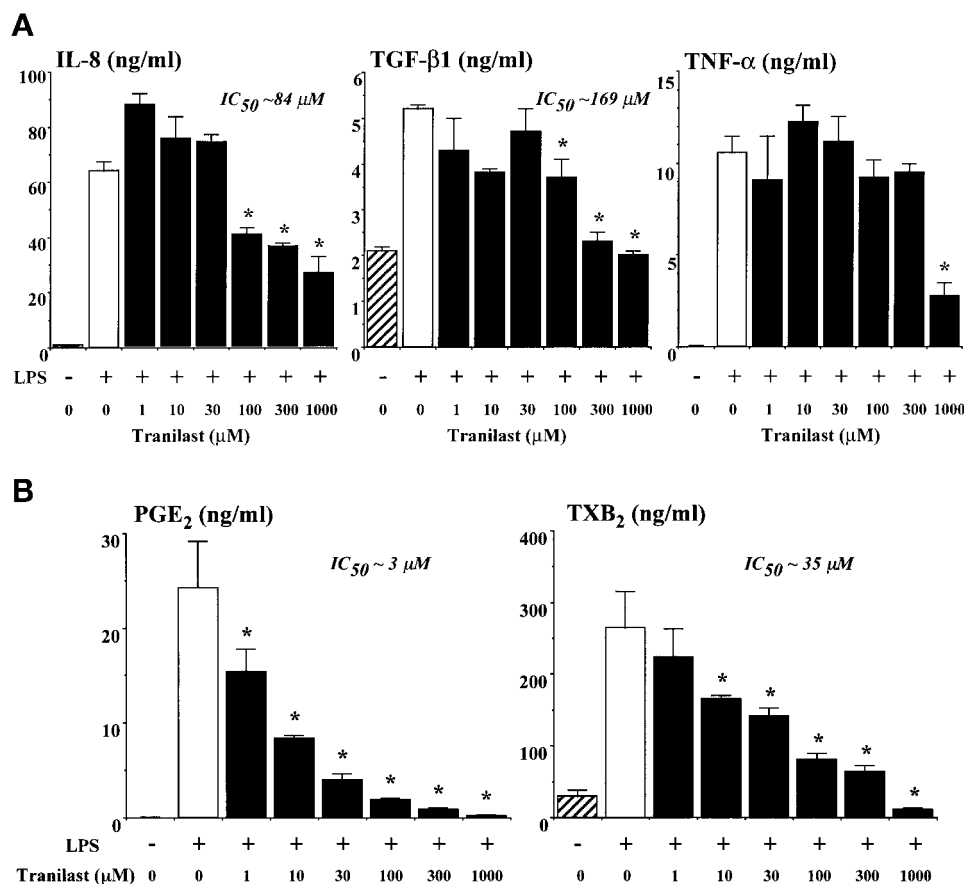
**Immunoblot Analysis.** Subcellular fractions were prepared as previously described (Marshall et al., 1997) from human monocytes yielding a 100,000g supernatant (cytosol, containing TGF-β1) and particulate fraction (microsomes, containing COX-II) and these were used to evaluate the effect of tranilast on cellular protein levels. Proteins were resolved by SDS-PAGE on 10% polyacrylamide gels (Bio-Rad, Hercules, CA) and transferred to nitrocellulose (Hybond-ECL, Amersham, Arlington Heights, IL). Immunoblot assays were performed and immunoreactive proteins were detected using the ECL Western blotting system (Amersham). Data were evaluated using scanning densitometry. Polyclonal antisera generated against human COX-II was produced by David DeWitt (Michigan University, East Lansing, MI).

**Statistical Analysis.** All studies were performed using two to six human donors. Data are expressed as mean  $\pm$  S.D. ( $n = 3$  determinations) and analyzed where indicated using the Student's *t* test for independent variables ( $P > .05$ ).

## Results

### Effect of Tranilast on the Function of Monocyte-Enriched Human PBMCs

Cytokine production was induced in monocyte-enriched human PBMCs by 24-h exposure to endotoxin (LPS) as de-



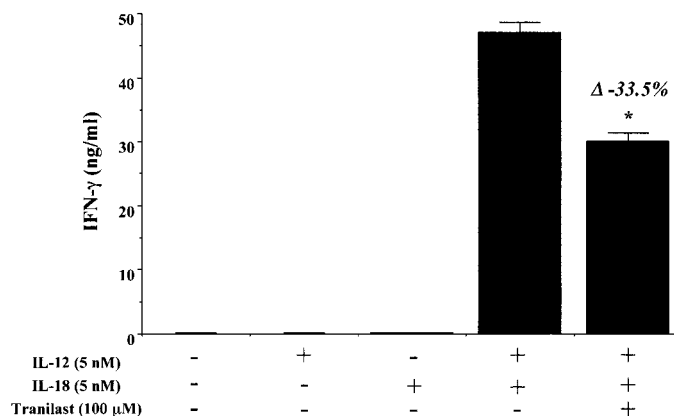
**Fig. 1.** Tranilast selectively inhibits inflammatory mediator release from LPS-stimulated monocyte-enriched human PBMCs. Cells were collected as described under *Materials and Methods* and were cultured ( $2 \times 10^6$ /ml) with and without stimulation by 200 ng/ml LPS for 24 h. Tranilast was delivered in 100% DMSO to a final concentration of 0.5%. Cell-free supernatants were analyzed by ELISA. A, release of IL-8, TNF- $\alpha$ , and TGF- $\beta$ 1 from one representative donor. B, release of PGE<sub>2</sub> and TXB<sub>2</sub> from one representative donor. All experiments were conducted in two to six donors. Data are expressed as nanograms per milliliter released, mean  $\pm$  S.D. ( $n = 3$ ), \* $P < .05$  compared with LPS stimulation alone. Toxicity was determined by Trypan blue exclusion.

scribed under *Materials and Methods* and the effect of varying concentrations (10–1000  $\mu$ M) of tranilast was evaluated. Toxicity by tranilast was noted ranging from 20 to 40% at 300  $\mu$ M to 40 to 60% at the highest dose, 1 mM. Figure 1A demonstrates that tranilast exhibited no significant inhibition of TNF- $\alpha$  production at nontoxic doses. In contrast, TGF- $\beta$ 1 ( $IC_{50} = \sim 100$ – $200 \mu$ M) and IL-8 production ( $IC_{50} = \sim 100 \mu$ M) were all reduced by tranilast in a concentration-dependent manner. Consistent with other published reports PGE<sub>2</sub> levels (Fig. 1B) were also reduced in a concentration-dependant manner ( $IC_{50} = \sim 1$ – $20 \mu$ M). We also saw decreases in induced thromboxane B<sub>2</sub> (TXB<sub>2</sub>) ( $IC_{50}$  values =  $\sim 10$ – $50 \mu$ M) levels after exposure to tranilast (Fig. 1B). This suggests inhibition of prostanoid metabolism by tranilast was not restricted to PGE<sub>2</sub> formation.

To evaluate the ability of tranilast to modulate a more selective immune-mediated response, monocyte-enriched human PBMCs were isolated as described under *Materials and Methods* and cells were incubated with either IL-12, IL-18, or both in the presence or absence of 100  $\mu$ M tranilast. Figure 2 shows, as previously described (Munder et al., 1998; Yoshimoto et al., 1998), exposure to either cytokine had no effect on IFN- $\gamma$  release, whereas coculture of these cells with both IL-12 and IL-18 (5 nM) results in the substantial release of IFN- $\gamma$ . Addition of tranilast to this system was able to significantly inhibit the induction of IFN- $\gamma$  by 33.5% (Fig. 2). IFN- $\gamma$  release after stimulation of PBMCs with higher concentrations of the cytokines (10 nM) was also inhibited by tranilast (data not shown).

**Studies on Tranilast Mechanism of Cellular Eicosanoid Modulation**

**Addition of Exogenous AA Substrate.** In an attempt to elucidate the mode of PGE<sub>2</sub> inhibition, LPS-stimulated monocyte-enriched human PBMCs were cultured with or without 20  $\mu$ M AA for 24 h. This would override the need for



**Fig. 2.** Tranilast inhibits IFN- $\gamma$  release by cytokine-stimulated monocyte-enriched human PBMCs. Cells were collected as described under *Materials and Methods* and were cultured ( $2 \times 10^6$ /ml) for 24 h with 5 nM IL-12 and IL-18, either alone or in combination, with or without 100  $\mu$ M tranilast. Tranilast was delivered in 100% DMSO to a final concentration of 0.5%. Cell-free supernatants were analyzed by ELISA. Shown is a graph from one representative donor. All experiments were conducted in two to six donors. Data are expressed as nanograms per milliliter release, mean  $\pm$  S.D. ( $n = 3$ ), \* $P < .05$  compared with IL-12/IL-18 stimulation alone. Toxicity was determined by Trypan blue exclusion.

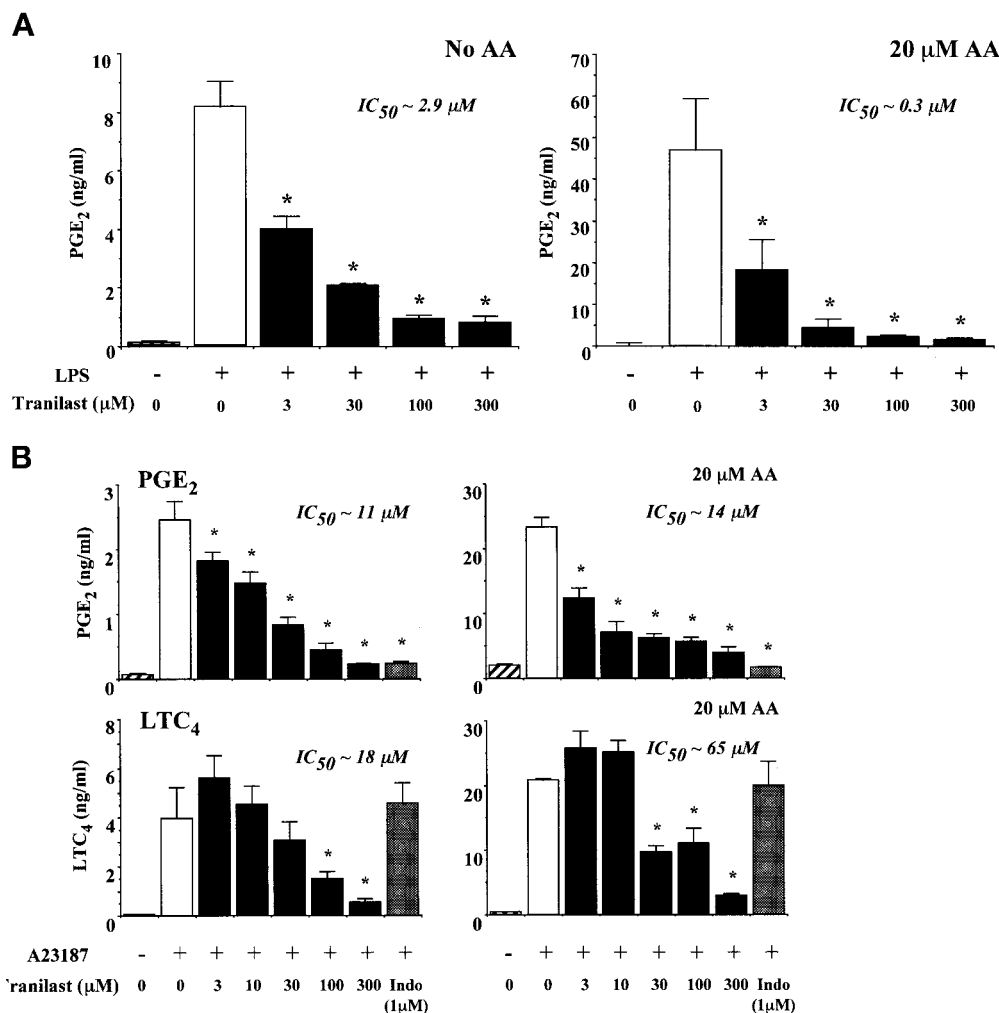
endogenous AA liberation via PLA<sub>2</sub> by providing the COX-II enzyme with substrate directly. LPS-stimulated cells exposed to AA produced 5 times more PGE<sub>2</sub> than LPS-stimulated cells alone, as expected (Fig. 3A). Tranilast potently inhibited PGE<sub>2</sub> production (IC<sub>50</sub> = ~2 μM in two donors) in the absence of exogenous AA, as had been shown in Fig. 1. The ability of tranilast to inhibit PGE<sub>2</sub> was not altered by culturing cells in the presence of 20 μM AA.

Monocyte-enriched human PBMCs were also stimulated with the broadly activating calcium ionophore A23187 (1 μM) for 7 min, again, with and without exogenous AA. Both prostanoids and leukotrienes are made in Ca<sup>2+</sup> ionophore-stimulated cells with COX-I as the primary prostanoid-synthesizing enzyme. This differs from the LPS system where prostanoids are generated by the inducible COX-II enzyme (Marshall et al., 1997). Figure 3B illustrates the effect of tranilast on PGE<sub>2</sub> and LTC<sub>4</sub> formation induced by A23187. Again, cells exposed to exogenous AA produced 5 to 6 times more PGE<sub>2</sub> or LTC<sub>4</sub> than A23187-stimulated cells alone. Tranilast inhibited both A23187-induced PGE<sub>2</sub> (IC<sub>50</sub> = ~2–20 μM) and LTC<sub>4</sub> (IC<sub>50</sub> = ~20–40 μM) formation in concentration-dependent manners. Indomethacin (1 μM) was used as a control and inhibited PGE<sub>2</sub> by 85 to 90% but, as expected, had no effect on LTC<sub>4</sub> formation (Fig. 3B). Again, addition of AA had no significant effect on the ability of

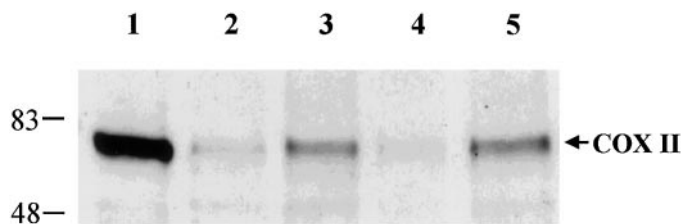
tranilast to inhibit A23187-induced PGE<sub>2</sub> (IC<sub>50</sub> = ~3–10 μM) or LTC<sub>4</sub> (IC<sub>50</sub> = ~30 μM) synthesis.

**Assessment of Inducible COX-II Expression.** COX-II protein levels were examined in 24 h LPS-stimulated monocyte-enriched human PBMCs cultured with or without tranilast (300 μM). Particulate fractions (100,000g) were prepared and analyzed by SDS-PAGE and Western blot as described under *Materials and Methods* (Fig. 4). As previously reported (Fu et al., 1990), little or no COX-II exists in untreated monocytes (Fig. 4, lane 2), whereas LPS stimulation over 24 h induced a significant up-regulation of protein (Fig. 4, lane 3). Treatment of unstimulated monocytes with tranilast had no significant effect on the basal levels of COX-II protein (Fig. 4, lane 4). Addition of up to 300 μM tranilast, although clearly able to inhibit LPS-induced PGE<sub>2</sub> levels as shown in Fig. 1B, showed no inhibition of COX-II protein expression in LPS-stimulated cells (Fig. 4, lane 5).

**Enzymatic Analysis of Tranilast on Phospholipase A<sub>2</sub> and Cyclooxygenase-II Enzyme Activities.** To provide more insight on the possible mechanisms of action, tranilast was evaluated for activity against PLA<sub>2</sub> enzymes, which provide AA as a substrate for conversion to eicosanoids, and on the COX-I and -II enzymes, which catalyze the first step of prostanoid production. Figure 5B shows tranilast moderately inhibited rh-85-kDa PLA<sub>2</sub>, giving only an ~47% reduction of



**Fig. 3.** Addition of exogenous arachidonic acid does not abrogate tranilast-mediated inhibition of eicosanoid release from monocyte-enriched human PBMCs. Cells were cultured ( $2 \times 10^6$ /ml) with increasing concentrations of tranilast (1 μM–1 mM) with and without 20 μM exogenous AA followed by stimulation with 200 ng/ml LPS for 24 h (A) or 1 μM A23187 (B) for 7 min. Tranilast was delivered in 100% DMSO to a final concentration of 0.5%. Cell-free supernatants were analyzed by ELISA. Shown are graphs from one representative donor. All experiments were conducted in two to six donors. Data are expressed as nanograms per milliliter release, mean  $\pm$  S.D. ( $n = 3$ ), \* $P < .05$  compared with stimulation alone. Toxicity was determined by Trypan blue exclusion.



**Fig. 4.** Tranilast has no effect on the expression levels of COX-II protein in LPS-stimulated monocyte-enriched human PBMCs. Cells ( $2 \times 10^6$ /ml) were cultured for 24 h with (lanes 3 and 5) or without 200 ng/ml LPS stimulation (lanes 2 and 4) in the presence (lanes 4 and 5) or absence (lanes 2 and 3) of tranilast at 300  $\mu$ M. Tranilast was delivered in 100% DMSO to a final concentration of 0.5%. Particulate fractions were resolved by SDS-PAGE (50  $\mu$ g/lane) and analyzed by Western blot for COX-II levels. Lane 1 is a COX-II standard. Shown is a blot from one representative donor. Experiments were conducted in two to four donors.

activity at 500  $\mu$ M. Although this *in vitro* inhibition reached statistical significance, the concentration of tranilast was 50-fold higher than the substrate-transition site 85-kDa PLA<sub>2</sub> inhibitor AACOCF<sub>3</sub> (IC<sub>50</sub> = ~10  $\mu$ M) (Lehr, 1997) and 10-fold higher than the concentrations needed to inhibit eicosanoid production in activated cells; therefore, it seems highly unlikely that this inhibition would be physiologically meaningful. Tranilast showed no effect on the *in vitro* *sn*-2 acylhydrolytic activity of rh-type IIA 14-kDa PLA<sub>2</sub> (Fig. 5A).

The ability of tranilast to affect prostanoid production by directly inhibiting cyclooxygenase activity was addressed in two separate *in vitro* assays. At concentrations as high as 1000  $\mu$ M tranilast showed no inhibition of either COX-I or -II activity. In the oxygen electrode assay (Laneville et al., 1994), microsomal preparations of COS-1 cells overexpressing COX-I (Fig. 6, triangles) or COX-II (Fig. 6, squares) enzymes were incubated with increasing concentrations of tranilast (Fig. 6, closed symbols) or flurbiprofen (Fig. 6, open symbols), a nonspecific COX inhibitor. Tranilast had no effect on the ability of either enzyme to oxygenate arachidonic acid, even at doses as high as 1 mM (Fig. 6). This is in contrast to the flurbiprofen-treated microsomal preparations. Both cyclooxygenase enzymes were potently inhibited by flurbiprofen in a dose-dependent manner (Fig. 6). The possibility of time-dependent inhibition was addressed as well. Preincubation of rh-COX-I- and -II-enriched microsomes with tranilast had no effect on enzyme activity in the oxygen electrode assay (data not shown). In all assays the incorporation of oxygen was dependent on the addition of arachidonic acid (data not shown). Tranilast activity was also evaluated in the more sensitive, whole-cell cyclooxygenase assay. COS-1 cells expressing either rh-COX-I or COX-II were preincubated with either vehicle or compound for 5 min before the addition of <sup>14</sup>C-labeled AA substrate. After 10 min the supernatants were extracted and examined for radiolabeled prostanoids by thin-layer chromatography. Table 1 shows tranilast, up to 200  $\mu$ M, had no significant effect on the ability of either the rh-COX-I or COX-II-transfected whole cells to make PGE<sub>2</sub>, PGD<sub>2</sub>, or PGF<sub>2 $\alpha$</sub> . In this assay treatment with flurbiprofen (10  $\mu$ M) resulted in 80 to 90% inhibition of both enzymes.

#### Further Evaluation of Tranilast Action on Monocyte TGF- $\beta$ 1 Formation

Immunoblot analysis of TGF- $\beta$ 1 was performed on cytosolic fractions of monocyte-enriched human PBMCs stimulated with LPS over 24 h with or without tranilast (300  $\mu$ M) as

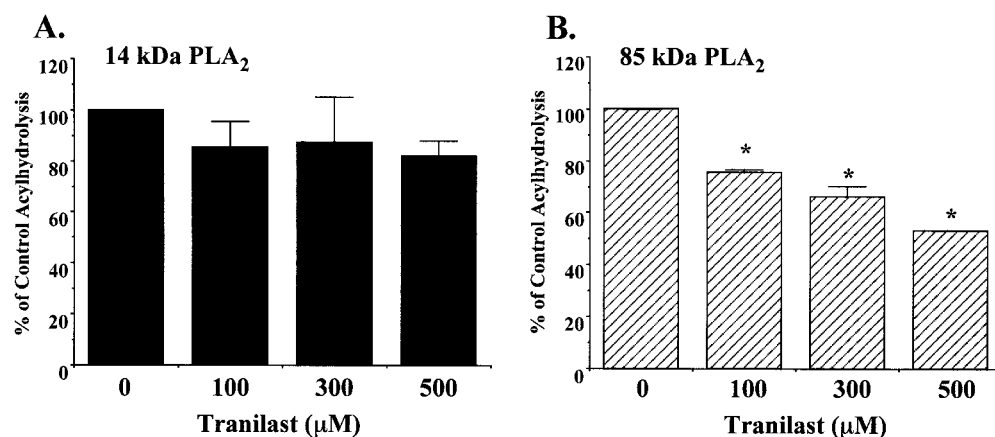
described under *Materials and Methods*. Untreated monocytes expressed TGF- $\beta$ 1 protein (Fig. 7, lane 1) most likely induced by adherence of the monocytes to the culture plate. LPS stimulation induced, on average, a 2-fold increase in the TGF- $\beta$ 1 levels over 24 h, as assessed by densitometry (Fig. 7, lane 2). Tranilast (300  $\mu$ M) treatment did not affect protein levels in unstimulated cells (Fig. 7, lane 3) but did reduce the production of TGF- $\beta$ 1 in LPS-stimulated cells to levels near or below those expressed by unstimulated monocytes (Fig. 7, lane 4). Northern analysis was performed on one donor in a parallel experiment and the results mirrored that seen in the Western blots (data not shown). TGF- $\beta$ 1 mRNA was present in unstimulated cells and tranilast was found to have no effect on these basal levels. LPS-stimulation produced a 2-fold up-regulation of TGF- $\beta$ 1 mRNA. This increase was almost totally blocked by treatment of the cells with 300  $\mu$ M tranilast.

**Effects of Exogenous PGE<sub>2</sub> on TGF- $\beta$ 1 Production in PBMCs.** To evaluate the possibility that the PGE<sub>2</sub> produced in response to activation may play a role in regulation of TGF- $\beta$ 1 formation, monocyte-enriched human PBMCs were stimulated with LPS and cultured in the presence of tranilast (300  $\mu$ M) with or without exogenous PGE<sub>2</sub> (2–200 ng/ml). The levels of the PGE<sub>2</sub> used were up to 10 times that measured in the conditioned media from LPS-activated PBMCs in the previous assays. Figure 8 shows the TGF- $\beta$ 1 measurements of one representative donor. Tranilast (300  $\mu$ M) inhibited TGF- $\beta$ 1 levels as expected. Addition of 200 ng/ml exogenous PGE<sub>2</sub> to the culture media was able to reverse the tranilast-mediated inhibition of TGF- $\beta$ 1 release. Complete restoration of TGF- $\beta$ 1 release was seen at concentrations of PGE<sub>2</sub> as low as 20 ng/ml (data not shown). Together, the data suggest an important role for PGE<sub>2</sub> in the mechanisms behind tranilast modulation of TGF- $\beta$ 1 output.

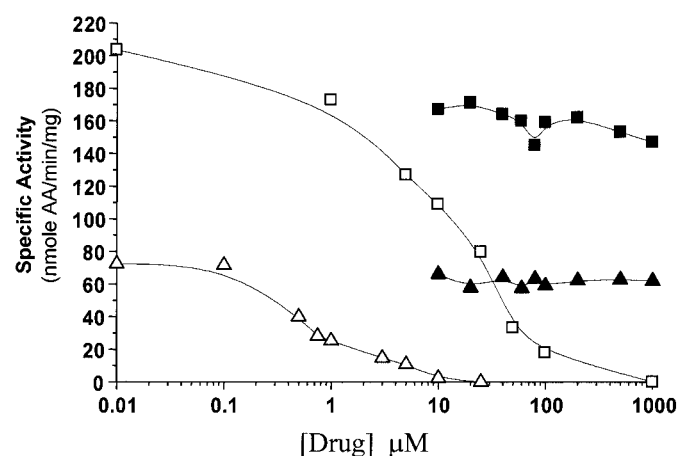
## Discussion

Tranilast is currently used as an antiallergic agent due to its potent inhibition of mast cell degranulation (Komatsu et al., 1988a). Tranilast possesses other immunomodulatory activities, such as inhibition of major histocompatibility complex class II expression (Kawano and Noma, 1993; Matsumura et al., 1999), but one of its most potent activities is reduction of eicosanoid synthesis (Komatsu et al., 1988b). Tranilast inhibits PDGF-induced proliferation and TGF- $\beta$ 1-mediated collagen deposition (Suzawa et al., 1992a; Miyazawa et al., 1995). Additional studies show it has other significant effects, which make it an attractive potential therapeutic for restenosis (Miyazawa et al., 1997; Ward et al., 1998). Here we make a more in depth evaluation of tranilast immune modulatory activities and attempt to elucidate or define its mechanism of action. In addition, we provide evidence that tranilast efficacy as a therapeutic agent in restenosis stems, in part, from its immunomodulatory activity in conjunction with its reported direct effects on proliferation (Tanaka et al., 1994; Nie et al., 1996).

In studies evaluating tranilast effects on the function of human monocytes, we confirm tranilast is a potent inhibitor of PGE<sub>2</sub> and LTC<sub>4</sub> release (IC<sub>50</sub> = 1–40  $\mu$ M) (Komatsu et al., 1988b). Prostanoid inhibition is not specific for PGE<sub>2</sub> because tranilast also inhibits TXB<sub>2</sub> with IC<sub>50</sub> values in the same range as those seen for PGE<sub>2</sub> release. Moreover, these data



**Fig. 5.** Tranilast does not significantly affect the *sn*-2 acylhydrolytic activity of recombinant human phospholipase A<sub>2</sub>. Tranilast was assessed for its effect on rh-type IIA (A) or rh-85-kDa PLA<sub>2</sub> (B). Enzymes were incubated with compound or vehicle before the addition of <sup>3</sup>H-*E. coli* substrate. Liberation of tritiated arachidonic acid was analyzed as described under *Materials and Methods*. Data are presented as percentage of control hydrolysis; \**P* < .05.



**Fig. 6.** Tranilast does not effect the activity of recombinant human COX-I or -II in transfected COS-1 cell microsomal preparations. Microsomal preparations of COS-1 cells transfected with rh-COX-I (▲, △) or -II (■, □) were incubated with increasing concentrations of tranilast (▲, ■) or flurbiprofen (△, □). Incorporation of oxygen onto the arachidonic acid substrate was evaluated via oxygen electrodes as described under *Materials and Methods*. Data are represented as the average specific activity as measured by the nanomoles of arachidonic acid oxygenated per minute per milligram of protein. In the absence of compounds specific activities were, on average, 68 nmol of AA/min/mg for COX-I and 180 nmol of AA/min/mg for COX-II. No oxygen consumption was detected in the absence of added arachidonate.

suggest tranilast exerts equivalent effects on both the cyclooxygenase and lipoxygenase pathways or perhaps interferes with a common pathway.

In an attempt to elucidate a mechanism for eicosanoid reduction, tranilast activity against several recombinant human enzymes important in the arachidonic acid cascade were evaluated. Tranilast had no effect on the *in vitro* activity of rh-type IIA PLA<sub>2</sub> and showed slight but not physiologically significant activity against the rh-85-kDa cytosolic PLA<sub>2</sub>. The lack of direct effect on *sn*-2 acylhydrolytic activity was further supported when, in cell assays using two different stimulatory mechanisms, LPS or ionophore, the addition of exogenous AA substrate was unable to abrogate the inhibitory effects of tranilast on eicosanoid release (Fig. 3, A and B). Taken together, these data suggest tranilast could act downstream of AA release. We show tranilast was ineffective at inhibiting rh-COX-I and -II enzyme activity either measured directly, using O<sub>2</sub> uptake, or through evaluation of prostaglandin production by cells transfected with recombinant COX-I or -II enzyme. This is in line with previous

reports assessing tranilast activity on prostanoid-synthesizing enzymes in microsomal preparations from rat peritoneal exudate cells (Komatsu et al., 1988b).

Tranilast was recently reported to reduce COX-II-like immunoreactivity in IL-1β-stimulated fibroblasts (Inoue et al., 1997). In the studies reported here, Western blot analysis of monocyte-enriched human PBMCs activated by LPS showed COX-II levels to be unaffected by tranilast treatment. This discrepancy in tranilast-mediated regulation of COX-II expression could be accounted for by differences in cell type, stimulation protocols, or antibody affinity. The previously published work (Inoue et al., 1997) used an anti-mouse COX-II antibody reported to cross-react with human isoforms, whereas our antibody was raised specifically against human COX-II, the enzyme under investigation in our studies. Nonetheless, in our hands, tranilast did not effect COX-II expression.

The ability of tranilast to affect eicosanoid release from PBMCs without directly affecting enzyme activity still leaves the question of mechanism. It has been shown the activity of eicosanoid-processing enzymes can be regulated at many levels, including transcription (Roshak et al., 1996) or phosphorylation (Lin et al., 1993) and, although not specifically addressed here, might be sites for tranilast intervention. Indeed, in preliminary studies using reporter assays the effect of tranilast on nuclear factor-κB activation has been inconclusive (data not shown), however an in-depth investigation into the ability of tranilast to modulate nuclear factor-κB activation remains to be completed.

Tranilast has also been shown to affect calcium influx in a number of different cell types (Komatsu et al., 1988a; Nie et al., 1997). The importance of calcium mobilization to inflammatory processes is well documented. Many of the enzymes involved in arachidonic acid release and eicosanoid biosynthesis require Ca<sup>2+</sup> for activation (Marshall and McCarter-Roshak, 1992) and/or translocation to substrate-rich membranes (Clark et al., 1991; Peters-Golden and McNish, 1993). The processes of exocytosis also require Ca<sup>2+</sup>-dependent membrane fusion and evidence suggests tranilast may perturb intercellular calcium equilibrium and hence degranulation via interactions with specific Ca<sup>2+</sup>-binding proteins (Shishibori et al., 1999). The ability of tranilast to cumulatively block the calcium-mediated activities necessary for eicosanoid biosynthesis is a potential mechanism that must still be explored.

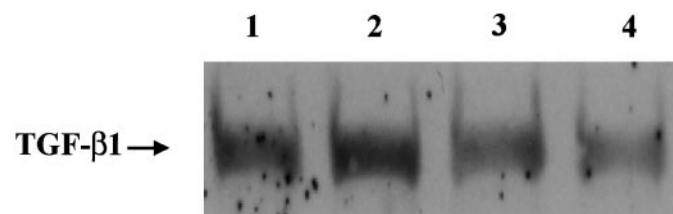
In addition to eicosanoid regulation, we show, for the first

TABLE 1

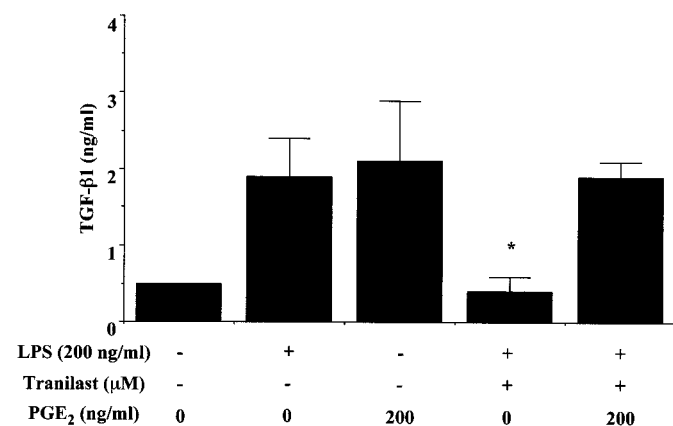
Tranilast does not inhibit prostaglandin synthesis by rh-COX enzymes in transfected COS-1 cells

Values given are the percentage of total radiolabeled product generated by COS-1 cells transfected with COX-I or -II after incubation with 10  $\mu\text{M}$  [ $1\text{-}^{14}\text{C}$ ]AA. Assays in intact cells were carried out as described under *Materials and Methods*. Prior to the addition of radiolabeled substrate, the cells were incubated with either vehicle, flurbiprofen (10  $\mu\text{M}$ ), or tranilast (50 and 200  $\mu\text{M}$ ). Reactions were terminated after 10 min at 37°C and supernatants were extracted and subjected to TLC.

	Drug	% Product			% Inhibition PG Synthesis
		PGE <sub>2</sub>	PGD <sub>2</sub>	PGF <sub>2<math>\alpha</math></sub>	
COX-I	No inhibitor	3.13	21.12	18.83	0
	10 $\mu\text{M}$ flurbiprofen	0.6	0.25	0.094	83
	50 $\mu\text{M}$ tranilast	3.42	21.07	19.83	-5
	200 $\mu\text{M}$ tranilast	6.57	24.07	18.16	-8
		12.77	47.12	22.73	0
COX-II	No inhibitor	12.77	47.12	22.73	0
	10 $\mu\text{M}$ flurbiprofen	1.57	3.62	3.37	86
	50 $\mu\text{M}$ tranilast	12.55	47.4	22.6	0
	200 $\mu\text{M}$ tranilast	9.22	43.98	19.17	10



**Fig. 7.** Tranilast exposure down-regulates the intracellular expression of TGF- $\beta$ 1 protein in human monocyte-enriched PBMCs. Cells ( $2 \times 10^6/\text{ml}$ ) were cultured for 24 h with (lanes 2 and 4) or without 200 ng/ml LPS stimulation (lanes 1 and 3) in the presence (lanes 3 and 4) or absence (lanes 1 and 2) of tranilast at 300  $\mu\text{M}$ . Tranilast was delivered in 100% DMSO with a resulting assay concentration of 0.5%. Particulate fractions (100,000g) were resolved by SDS-PAGE (35  $\mu\text{g}/\text{lane}$ ) and analyzed by Western blot for TGF- $\beta$ 1 levels. Shown is a blot from one representative donor. Experiments were conducted in two to four donors.



**Fig. 8.** Addition of exogenous PGE<sub>2</sub> can reverse the tranilast-mediated inhibition of TGF- $\beta$ 1 release in human monocyte-enriched PBMCs. Cells ( $2 \times 10^6/\text{ml}$ ) were cultured for 24 h with 200 ng/ml LPS stimulation in the presence or absence of tranilast at 300  $\mu\text{M}$ . PGE<sub>2</sub> (200 ng/ml) was added to the media. Tranilast was delivered in 100% DMSO to a final concentration of 0.5%. Cell-free supernatants were analyzed for TGF- $\beta$ 1 by ELISA. Shown is a graph from one representative donor. All experiments were conducted in two to six donors. Data are expressed as nanograms per milliliter release, mean  $\pm$  S.D. ( $n = 3$ ). \* $P < .05$  compared with stimulation alone.

time, tranilast's ability to modulate LPS-induced PBMC IL-8, a potent chemoattractant and activator of neutrophils. Although this required higher levels, inhibition was concentration-dependent ( $\text{IC}_{50} = \sim 100 \mu\text{M}$ ). The ability of tranilast to lower IL-8 levels at the site of tissue damage would, in theory, augment the tissue repair profile through a reduced granulocyte influx. Interestingly, tranilast had no effect on monocyte TNF- $\alpha$  release, indicating the lack of a general

immunotoxic effect and suggesting a distinct mechanism(s) of action.

Macrophage responsiveness at wound sites would, potentially, also be effected by tranilast via the attenuation of IFN- $\gamma$ , the most potent activator of macrophages. In a cytokine activation system, combinatorial treatment with IL-12 and IL-18 drives mononuclear cells to produce IFN- $\gamma$ . Tranilast was an inhibitor in this system (33.5% at 100  $\mu\text{M}$ ). This would presumably attenuate the IFN- $\gamma$  activation loop, reducing overall macrophage responsiveness during an immune-mediated response.

Human monocytes are known to be an important source of the growth factor TGF- $\beta$ 1 (Letterio and Roberts, 1998). TGF- $\beta$ 1 is a key regulator of matrix deposition, which directly impacts tissue repair and remodeling (Derynck, 1994). Tranilast has been shown to inhibit the release of TGF- $\beta$ 1 in a number of species and cell systems (Suzawa et al., 1992b; Ward et al., 1998). Its ability to prevent keloid formation correlates to reduced TGF- $\beta$ 1-induced extracellular matrix deposition by fibroblasts (Suzawa et al., 1992a; Yamada et al., 1994). Several animal models of hyperproliferation and fibrosis, where TGF- $\beta$ 1 activity is deemed instrumental, have seen attenuation by treatment with tranilast (Isaji et al., 1994; Mori et al., 1995), including several models of vascular restenosis (Kikuchi et al., 1996; Miyazawa et al., 1997; Ward et al., 1998).

Initial reports of tranilast-mediated TGF- $\beta$ 1 reduction in mononuclear cells was shown not to be concentration-dependent (Suzawa et al., 1992b) but this may have been due to the use of an overwhelming amount of stimulus (i.e., LPS at 1 mg/ml). We show here that tranilast reduces TGF- $\beta$ 1 levels in a concentration-dependent manner ( $\text{IC}_{50}$  values =  $\sim 100$ –200  $\mu\text{M}$ ) in LPS-stimulated monocytes (LPS at 200 ng/ml; a dose lying on the linear portion of a concentration versus activation curve). In agreement with this we show, for the first time, that intracellular protein levels of TGF- $\beta$ 1 are reduced in monocytes treated with tranilast (Fig. 7). Preliminary Northern analyses suggest this inhibition is transcriptional although the exact mechanisms by which tranilast specifically attenuates TGF- $\beta$ 1 expression are not known. However, tranilast's lack of effect on COX-II expression and its inability to attenuate TNF- $\alpha$  levels suggests specific mechanistic activities distinct from those of a general transcriptional inactivator. Because the monocyte/macrophage is a key source of TGF- $\beta$ 1 at sites of injury and inflammation, attenuation of TGF- $\beta$ 1 formation would impact heavily on over-reactive tissue repair processes.

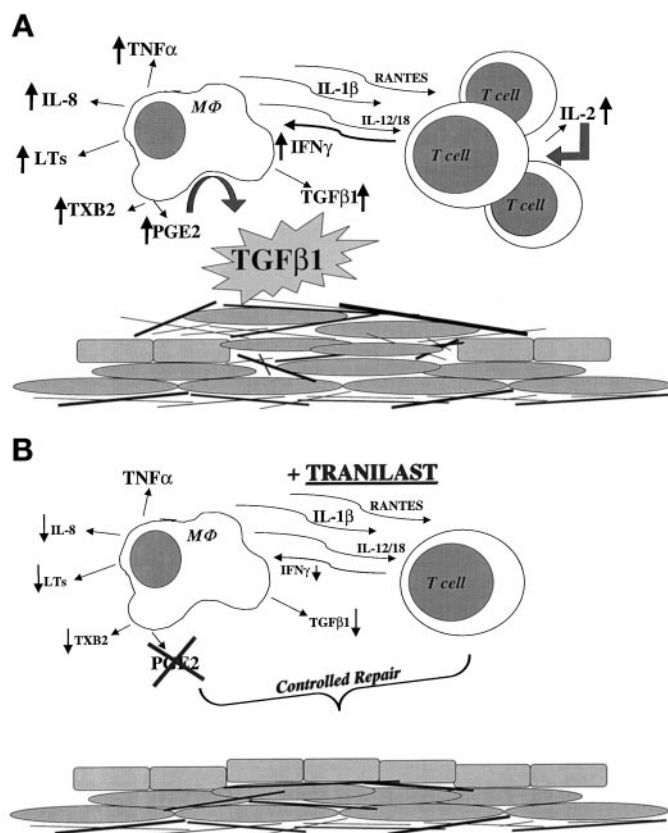
During an inflammatory response several chemical mediators are produced, each with the potential to impact the others. We observed addition of PGE<sub>2</sub> to LPS-stimulated monocytes treated with tranilast abrogated the tranilast-induced TGF-β1 reduction. This suggests that TGF-β1 inhibition is secondary to tranilast-mediated reduction of PGE<sub>2</sub> and is consistent with the 10-fold less potent activity observed for TGF-β1 compared with PGE<sub>2</sub>.

From the data presented here, one could hypothesize a model of immune-mediated fibrosis and its subsequent attenuation by tranilast (Fig. 9). After tissue damage phagocytic cells are drawn in and activated. Subsequent release of chemical mediators results in the recruitment of additional inflammatory cells, i.e., T cells. Monocyte activation is perpetuated by local increases in IFN-γ. Production of PGE<sub>2</sub> by activated inflammatory cells initiates prolonged increases in TGF-β1 levels, leading to cellular migration, proliferation, and increased extracellular matrix deposition (Fig. 9A). Once released, TGF-β1 has been shown to participate in an auto-amplification regulatory loop, thereby enhancing its own production and hence prolonging inflammation and fibrosis long past the initiating event (Derynck, 1994; Anderson et al., 1996). Early intervention, with tranilast as a therapeutic agent, would effectively block the inflammatory cascade at multiple levels, i.e., gene expression and mediator release (Fig. 9B). Reductions in cytokine production would attenuate

the influx of additional immune cells. Interferon-γ inhibition would stop the continued activation of proinflammatory cascades, whereas blockade of PGE<sub>2</sub> production would impact the production of TGF-β1. As a consequence, we believe the efficacy of tranilast in preventing fibrosis and restenosis stems from a synergy between its immunomodulatory and antiproliferative activities, which culminate to attenuate aberrant tissue repair.

## References

- Anderson KM, Brawner ME and Nichols AJ (1996) Transforming growth factor-β in restenosis, in *Coronary Restenosis: From Genetics to Therapeutics* (Feuerstein GZ ed) pp 99–127, Marcel Dekker, New York.
- Azuma H, Banno K and Yoshimura T (1976) Pharmacological properties of N-(3',4'-dimethoxycinnamoyl) anthranilic acid (N-5'), a new anti-atopic agent. *Br J Pharmacol* **58**:483–488.
- Clark JD, Lin L-L, Kriz RW, Ramesha CS, Sultzman LA, Lin AJ, Milona N and Knopf JL (1991) A novel arachidonic acid selective cytosolic PLA<sub>2</sub> contains a Ca<sup>2+</sup>-dependent translocation domain with homology to PKC and GAP. *Cell* **65**:1043–1051.
- Derynck R (1994) Transforming growth factor-beta, in *The Cytokine Handbook* (Thomson AW ed) pp 319–342, Academic Press, London.
- Fu JY, Masferrer JL, Seibert K, Raz A and Needleman P (1990) The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes. *J Biol Chem* **265**:16737–16740.
- Gruntzig A (1978) Transluminal dilation of coronary-artery stenosis. *Lancet* **1**:263–263.
- Inoue H, Oshima H, Kono H, Yamanaka M, Kubota T, Aihara M, Hiroi T, Yago N and Ishida H (1997) Suppressive effects of tranilast on the expression of inducible cyclooxygenase (COX2) in interleukin-1β-stimulated fibroblasts. *Biochem Pharmacol* **53**:1941–1944.
- Isaji M, Aruga N, Naito J and Miyata H (1994) Inhibition by tranilast of collagen accumulation in hypersensitive granulomatous inflammation in vivo and for morphological changes and functions of fibroblasts in vitro. *Life Sci* **55**:287–292.
- Kawano Y and Noma T (1993) Cell action mechanism of tranilast - effect on the expression of HLA-class II antigen. *Int J Immunopharmacol* **15**:487–500.
- Kikuchi S, Umemura K, Kondo K and Nakashima M (1996) Tranilast suppresses intimal hyperplasia after photochemically induced endothelial injury in the rat. *Eur J Pharmacol* **295**:221–227.
- Komatsu H, Kojima M, Tsutsumi N, Hamano S, Kusama H, Ujiie A, Ikeda S and Nakazawa M (1988a) Study of the mechanism of inhibitory action of tranilast on chemical mediator release. *Jpn J Pharmacol* **46**:43–51.
- Komatsu H, Kojima M, Tsutsumi N, Hamano S, Kusama H, Ujiie A, Ikeda S and Nakazawa M (1988b) Mechanism of inhibitory action of tranilast on the release of slow reacting substance of anaphylaxis (SRS-A) in vitro: Effect of tranilast on the release of arachidonic acid and its metabolites. *Jpn J Pharmacol* **46**:53–60.
- Laneville O, Breuer DK, Dewitt DL, Hla T, Funk CD and Smith WL (1994) Differential inhibition of human prostaglandin endoperoxide H synthases-1 and -2 by nonsteroidal anti-inflammatory drugs. *J Pharmacol Exp Ther* **271**:927–934.
- Lehr M (1997) Structure-activity relationships of (4-Acylpyrrol-2-yl)alkanoic acids as inhibitors of the cytosolic phospholipase A<sub>2</sub>: Variation of the substituents in positions 1,3, and 5. *J Med Chem* **40**:3381–3392.
- Letterio JJ and Roberts AB (1998) Regulation of immune responses by TGF-β. *Annu Rev Immunol* **16**:137–161.
- Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A and Davis RJ (1993) cPLA<sub>2</sub> is phosphorylated and activated by MAP kinase. *Cell* **72**:269–278.
- Marshall LA, Bauer J, Sung ML and Chang JY (1991) Evaluation of antirheumatic drugs for their effect in vitro on purified human synovial fluid phospholipase A<sub>2</sub>. *J Rheumatol* **18**:59–65.
- Marshall LA, Bolognese B, Winkler JD and Roshak A (1997) Depletion of human monocyte 85-kDa phospholipase A<sub>2</sub> does not alter leukotriene formation. *J Biol Chem* **272**:759–765.
- Marshall LA and McCarte-Roshak A (1992) Demonstration of similar calcium dependencies by mammalian high and low molecular mass phospholipase A<sub>2</sub>. *Biochem Pharmacol* **44**:1849–1858.
- Matsumura T, Kugiyama K, Sugiyama S, Ota Y, Doi H, Ogata N, Oka H and Yasue H (1999) Suppression of atherosclerotic development in Watanabe heritable hyperlipidemic rabbits treated with an oral antiallergic drug, tranilast. *Circulation* **99**:919–924.
- Miyazawa K, Kikuchi S, Fukuyama J, Hamano S and Ujiie A (1995) Inhibition of PDGF- and TGF-β1-induced collagen synthesis, migration and proliferation by tranilast in vascular smooth muscle cells from spontaneously hypertensive rats. *Atherosclerosis* **118**:213–221.
- Miyazawa N, Umemura K, Kondo K and Nakashima M (1997) Effects of pemirolast and tranilast on intimal thickening after arterial injury. *J Cardiovasc Pharmacol* **30**:157–162.
- Mori H, Kawada K, Zhang P, Uesugi Y, Sakamoto O and Koda A (1991) Bleomycin-induced pulmonary fibrosis in genetically mast cell deficient WBB6F<sub>1</sub>-W/W<sup>o</sup> mice and mechanism of the suppressive effect of tranilast, an antiallergic drug inhibiting mediator release from mast cells, on fibrosis. *Int Arch Allergy Appl Immunol* **95**:195–201.
- Mori H, Tanaka H, Kawada K, Nagai H and Koda A (1995) Suppressive effects of tranilast on pulmonary fibrosis and activation of alveolar macrophages in mice



**Fig. 9.** Schematic representation of tranilast activity in modulating immune-mediated wound repair. A, representation of the possible signaling events mediating the complex phenomena of aberrant tissue repair after PTCA, leading ultimately to restenosis. B, representation of how down-regulation of immune-mediated signaling cascades initiated by PTCA via therapeutic intervention with tranilast might allow for normal wound healing sequelae.



- treated with bleomycin: Role of alveolar macrophages in fibrosis. *Jpn J Pharmacol* **67**:279–289.
- Munder M, Mallo M, Eichmann K and Modolell M (1998) Murine macrophages secrete interferon- $\gamma$  upon combined stimulation with interleukin (IL)-12 and IL-18: A novel pathway of autocrine macrophage activation. *J Exp Med* **187**:2103–2108.
- Nie L, Oishi Y, Doi I, Shibata H and Kojima I (1997) Inhibition of proliferation of MCF-7 breast cancer cells by a blocker of Ca<sup>2+</sup>-permeable channel. *Cell Calcium* **22**:75–82.
- Nie L, Mogami H, Kanzaki M, Shibata H and Kojima I (1996) Blockade of DNA synthesis induced by platelet-derived growth factor by tranilast, an inhibitor of calcium entry, in vascular smooth muscle cells. *Mol Pharmacol* **50**:763–796.
- Peters-Golden M and McNish RW (1993) Redistribution of 5-lipoxygenase and cytosolic phospholipase A<sub>2</sub> to the nuclear fraction upon macrophage stimulation. *Biochem Biophys Res Commun* **196**:147–153.
- Roshak AK, Jackson JR, McGough K, Chabot-Fletcher M, Mochan E and Marshall LA (1996) Manipulation of distinct NF $\kappa$ B proteins alters interleukin-1 $\beta$ -induced human rheumatoid synovial fibroblast prostaglandin E<sub>2</sub> formation. *J Biol Chem* **271**:31496–31501.
- Ross R (1999) Atherosclerosis is an inflammatory disease. *Am Heart J* **138**:S419–S420.
- Serrano CV, Ramires JAF, Venturinelli M, Arie S, D'Amico E, Zweier JL, Pileggi F and da Luz PL (1997) Coronary angioplasty results in leukocyte and platelet activation with adhesion molecule expression: Evidence of inflammatory responses in coronary angioplasty. *J Am Coll Cardiol* **29**:1276–1283.
- Shishibori T, Oyama Y, Matsushita O, Yamashita K, Furuichi H, Okabe A, Maeta H, Hata Y and Kobayashi R (1999) Three distinct anti-allergic drugs, amlexanox, cromolyn and tranilast, bind to S100A12 and S100A13 of the S100 protein family. *Biochem J* **338**:583–589.
- Suzawa H, Kikuchi S, Arai N and Koda A (1992a) The mechanism involved in the inhibitory action of tranilast on collagen biosynthesis of keloid fibroblasts. *Jpn J Pharmacol* **60**:91–96.
- Suzawa H, Kikuchi S, Ichikawa K and Koda A (1992b) Inhibitory action of tranilast, an anti-allergic drug, on the release of cytokines and PGE<sub>2</sub> from human monocytes-macrophages. *Jpn J Pharmacol* **60**:85–90.
- Tamai H, Katoh O, Suzuki S, Fujii K, Aizawa T, Takase S, Kurogane H, Nishikawa H, Sone T, Sakai K and Suzuki T (1999) Impact of tranilast on restenosis after coronary angioplasty: Tranilast restenosis following angioplasty trial (TREAT). *Am Heart J* **138**:968–975.
- Tanaka K, Honda M, Kuramochi T and Morioka S (1994) Prominent inhibitory effects of tranilast on migration and proliferation of and collagen synthesis by vascular smooth muscle cells. *Atherosclerosis* **107**:179–185.
- Ward MR, Sasahara T, Agrotis A, Dilley RJ, Jennings GL and Bobik A (1998) Inhibitory effects of tranilast on expression of transforming growth factor- $\beta$  isoforms and receptors in injured arteries. *Atherosclerosis* **137**:267–275.
- Yamada H, Tajima S, Nishikawa T, Murad S and Pinnell SR (1994) Tranilast, a selective inhibitor of collagen synthesis in human skin fibroblasts. *J Biochem* **116**:892–897.
- Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, Akira S and Nakanishi K (1998) IL-12 upregulates IL-18 receptor expression on T cells, T<sub>H</sub>1 cells, and B cells: Synergism with IL-18 for IFN- $\gamma$  production. *J Immunol* **161**:3400–3407.

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