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Type IIA Secretory Phospholipase A$_2$ Up-Regulates Cyclooxygenase-2 and Amplifies Cytokine-Mediated Prostaglandin Production in Human Rheumatoid Synoviocytes

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Human type IIA secretory phospholipase A$_2$ (sPLA$_2$-IIA) is induced in association with several immune-mediated inflammatory conditions. We have evaluated the effect of sPLA$_2$-IIA on PG production in primary synovial fibroblasts from patients with rheumatoid arthritis (RA). At concentrations found in the synovial fluid of RA patients, exogenously added sPLA$_2$-IIA dose-dependently amplified TNF-$\alpha$-stimulated PGE$_2$ production by cultured synovial fibroblasts. Enhancement of TNF-$\alpha$-stimulated PGE$_2$ production in synovial cells was accompanied by increased expression of cyclooxygenase (COX)-2 and cytosolic phospholipase A$_2$ (cPLA$_2$)-$\alpha$. Blockade of COX-2 enzyme activity with the selective inhibitor NS-398 prevented both TNF-$\alpha$-stimulated and sPLA$_2$-IIA-amplified PGE$_2$ production without affecting COX-2 protein induction. However, both sPLA$_2$-IIA-amplified PGE$_2$ production and enhanced COX-2 expression were blocked by the sPLA$_2$ inhibitor LY311727. Colocalization studies using triple-labeling immunofluorescence microscopy showed that sPLA$_2$-IIA and cPLA$_2$-$\alpha$ are coexpressed with COX-2 in discrete populations of CD14-positive synovial macrophages and synovial tissue fibroblasts from RA patients. Based on these findings, we propose a model whereby the enhanced expression of sPLA$_2$-IIA by RA synovial cells up-regulates TNF-$\alpha$-mediated PG production via superinduction of COX-2. Therefore, sPLA$_2$-IIA may be a critical modulator of cytokine-mediated synovial inflammation in RA. The Journal of Immunology, 2000, 165: 2790–2797.

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammation of the joint synovium that results in pain, joint erosion, and dysfunction. The severity of joint inflammation fluctuates, resulting in exacerbations and remissions of disease activity. The cytokines, TNF-$\alpha$ and IL-1$\beta$, play an important role in the pathogenesis of RA. Neutralization of these cytokines alleviates synovial inflammation in both animal models and human RA (1). These animal studies, together with IL-1$\beta$ and TNF-$\alpha$ in vivo gene deletion experiments, have shown that IL-1$\beta$ is consistently important in mediating cartilage breakdown, whereas TNF-$\alpha$ is a key inducer of synovial inflammation (2). Moreover, transgenic mice overexpressing TNF-$\alpha$ develop spontaneous arthritis (3). TNF-$\alpha$ and IL-1$\beta$ activate the transcription factor NF-kB (4) and the p38 and c-Jun N-terminal kinase mitogen-activated protein kinase (MAPK) pathways (5) to induce a host of proinflammatory proteins. Direct inhibition of NF-kB (6), Jun/Fos (AP-1) (7) or p38 MAPK (8) reduces disease severity in animal models of arthritis, confirming the importance of these signaling pathways in the inflammatory and/or the erosive component of arthritis.

Prostaglandin E$_2$ (PGE$_2$) contributes to pain and swelling during inflammation through induction of hyperalgesia and increased vascular permeability (9) and modulates bone resorption through stimulation of osteoclast formation from precursor stem cells (10). PGE$_2$ production by cultured rheumatoid synovial fibroblasts (RSFs) is induced within hours by IL-1$\beta$ mediated by NF-kB- and MAPK-dependent coordinate induction of cytosolic phospholipase A$_2$ (cPLA$_2$)-$\alpha$ and cyclooxygenase (COX)-2 (11, 12). Although COX-1 is constitutively expressed by RSFs, IL-1$\beta$-stimulated PGE$_2$ production occurs exclusively via COX-2 (13). In RA synovium both COX-1 and -2 are expressed, with COX-2 expression elevated in relation to the degree of inflammation in synovial tissue (14). Recently, COX-2-selective inhibitors that maintain the anti-inflammatory properties of nonsteroidal anti-inflammatory drugs (NSAIDs) (15, 16) have been developed; but, unlike the latter compounds, they have a favorable gastrointestinal side effect profile.

A low molecular mass (14 kDa) human type IIA secretory phospholipase A$_2$ (sPLA$_2$-IIA) has been identified in rheumatoid synovium (17); however, the importance of this enzyme in synovial pathology is poorly defined. sPLA$_2$-IIA, first purified from the synovial fluid of patients with RA (18), is found at high levels in the colonic mucosa of patients with ulcerative colitis and Crohn’s disease (19), in the bronchoalveolar lavage fluid of patients with asthma following Ag challenge (20) and in the serum of septic shock patients (21). Serum sPLA$_2$-IIA concentrations are elevated in patients with RA (22) and correlate with severity of disease (23). Enzyme expression is increased in RA synovial macrophages and...
fibroblasts relative to synovium from nonarthritic patients and correlates with histological markers of synovial inflammation (17). Human sPLA₂-IIA is acutely inflammatory when injected into rabbit joints (24); however, transgenic mice overexpressing sPLA₂-IIA do not develop arthritis (25).

Given the presence of sPLA₂-IIA at concentrations up to several micrograms per milliliter in RA synovial fluid (26), and the established inflammatory activities of PGs (9), we have examined here both the relationship between sPLA₂-IIA and PGE₂ production in cultured RSFs and the cellular localization of sPLA₂-IIA and COX-2 in rheumatoid synovial tissue. The results of these studies demonstrate that concentrations of sPLA₂-IIA found in RA synovial fluid enhance TNF-α-stimulated PGE₂ production in RSFs by superinducing COX-2 protein levels. Further, sPLA₂-IIA and COX-2 colocalize in discrete subpopulations of rheumatoid synovial macrophages and fibroblasts. These findings indicate that sPLA₂-IIA may be an important amplifier of cytokine-mediated PG production and may thereby contribute to the severity of the synovial inflammatory response in RA.

Materials and Methods

Materials

sPLA₂-IIA was purified from the conditioned medium and cell pellets of a Chinese hamster ovary cell line (5A2) stably expressing human sPLA₂-IIA cDNA (27) by immunoadfinity chromatography on an AKTA explorer system (Pharmacia Biotech, Uppsala, Sweden) and quantified by ELISA (22). sPLA₂-IIA was a single 14-kDa band on silver-stained PAGE gels, and N-terminal amino acid sequence analysis (27) confirmed its identity. sPLA₂-IIA was contained <0.1 ng endotoxin/mg protein (Limplus amebocyte lysate pyrochrome, Associates of Cape Cod, Falmouth, MA) and was enzymatically active in a [³H]arachidonate-labeled Escherichia coli membrane assay (27).

Fibroblast culture

Synovial tissues were obtained as described (17) using procedures approved by the St. Vincent's Hospital Ethics Committee. RSFs were isolated by trypsin (0.5%)/EDTA (5.3 mM) digestion (15 min, 37°C in DMEM/Ham's F12 medium), followed by collagenase (200 U/ml; Life Technologies, Gaithersburg, MD), and cells were grown to confluence in medium containing 10% FBS (Commonwealth Serum Laboratory, Melbourne, Australia), penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (0.125 µg/ml) (Life Technologies), before storage in liquid nitrogen. Cells were phenotypically stable (CD14-negative, prolyl-5-hydroxylase-positive by immunofluorescence microscopy) to passage and received fresh medium containing 0.1% BSA (endotoxin-free, fatty acid-free; Boehringer Mannheim, Sydney, Australia) before stimulation.

PGE₂ studies

Inhibitors LY311727 (28) and NS-398 (29) (Cayman Chemical, Ann Arbor, MI) were prepared as 10-mM stocks in DMSO. Both control and inhibitor-treated cultures contained a final solution concentration of 0.1% (v/v) DMSO. LY311727 was inhibitory toward sPLA₂-IIA (IC₅₀, 2.5 µM at an sPLA₂-IIA concentration of 10 ng/ml; Escherichia coli membrane assay (27)), whereas NS-398 was noninhibitory up to 100 µM (data not shown). Confluent fibroblast monolayers were stimulated with sPLA₂-IIA, human rTNF-α (Peprotech, Rocky Hill, NJ), or IL-1β (R&D Systems, Minneapolis, MN) alone or in combination, in the presence or absence of inhibitors for 24 h, and media were stored at −80°C. PGE₂ was quantified in triplicate at three dilutions by enzyme immunoassay (Cayman Chemical).

Western blot analysis

Cell lysates were prepared by resuspension in PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, aprotinin (50 µg/ml), leupeptin (200 µM), and PMSF (1 mM) followed by repeated passage through a 21-gauge needle, incubation on ice (30 min), centrifugation (20 min, 13,800 x g, 4°C), and storage of supernatants at −80°C. Lysates were analyzed by Western blotting following SDS-PAGE (4–20% gradient gels; Novex, San Diego, CA). Primary Abs were anti-ovine COX-1 mAb (cat. no. 160110, 1.7 µg/ml; Cayman Chemical), anti-human COX-2 mAb (cat. no. 160112, 0.5 µg/ml; Cayman Chemical), anti-human cPLA₂-α mAb (cat. no. sc-454, 0.1 µg/ml; Santa Cruz Biotechnology, Santa Cruz, CA), anti-β-actin mAb (clone AC-15, 0.3 µg/ml; Sigma, St. Louis, MO), and anti-human ICAM-1 goat polyclonal Ab (cat. no. sc-1510, 0.2 µg/ml; Santa Cruz Biotechnology). Proteins were visualized using enhanced chemiluminescence (NEN, Boston, MA) and quantified by densitometry (Molecular Dynamics, Sunnyvale, CA).

Immunofluorescence and confocal microscopy

Synovial tissue from five RA patients receiving, alone or in combination, NSAIDs, auranofin, azathioprine, cyclosporin, sulfasalazine, or methotrexate, but not prednisolone, was examined by immunofluorescence microscopy as described (17). Sections were sequentially incubated with anti-human COX-2 goat polyclonal Ab (cat. no. sc-1745, 5 µg/ml, 45 min; Santa Cruz Biotechnology) or negative control goat IgG (5 µg/ml; Sigma), donkey anti-goat rhodamine red-X conjugate (1/100, 30 min; Jackson ImmunoResearch, West Grove, PA), a second primary Ab (45 min), and donkey anti-mouse-iododecarbocyanine (Cy5) conjugate (1/200, 30 min; Jackson ImmunoResearch). For double-labeling studies, second primary Abs were anti-human CD68 (clone EBM11, 4.3 µg/ml; Dako, Glostrup, Denmark). Abs were anti-human CD68 (clone EBM11, 4.3 µg/ml; Dako, Glostrup, Denmark).
sPLA$_2$-IIA AMPLIFICATION OF SYNOVIAL PG PRODUCTION

STATISTICAL ANALYSIS

Statistical evaluations were performed on primary data using the Wilcoxon signed rank test or the Student’s unpaired $t$ test with StatView software (Abacus Concepts, Berkeley, CA).

RESULTS

COX-2 mediates TNF-$\alpha$-stimulated PGE$_2$ production

To determine the responsiveness of the cPLA$_2$-COX pathway to TNF-$\alpha$, RSFs were stimulated with increasing concentrations of TNF-$\alpha$, then PGE$_2$ production and COX and cPLA$_2$-\alpha protein levels were determined. Basal PGE$_2$ production varied between cultures (range 46 ± 2 to 245 ± 10 pg/ml); however, in each of the five cultures, TNF-$\alpha$ dose-dependently stimulated PGE$_2$ production (range 0.32 ± 0.07 to 13.4 ± 3.1 ng/ml at 10 ng/ml TNF-$\alpha$) (Fig. 1A). Increased PGE$_2$ production correlated with accumulation of COX-2 protein (Fig. 1B). cPLA$_2$-\alpha was weakly but consistently up-regulated with COX-2, whereas COX-1 was unaffected. The adhesion molecule ICAM-1 was near-maximally induced at 1 ng/ml TNF-$\alpha$ (Fig. 1B).

Treatment of RSFs with TNF-$\alpha$ (10 ng/ml) in the presence of the COX-2-selective inhibitor NS-398 (1 \muM) (29) abrogated TNF-$\alpha$-stimulated PGE$_2$ production whereas the sPLA$_2$-selective inhibitor LY311727 (28) reproducibly suppressed PGE$_2$ by 30% (Fig. 2).

sPLA$_2$-IIA amplification of TNF-$\alpha$-stimulated PGE$_2$ production

RSFs were treated with increasing concentrations of sPLA$_2$-IIA in the presence or absence of TNF-$\alpha$ (10 ng/ml), sPLA$_2$-IIA alone did not stimulate PGE$_2$ production at any concentration examined (Fig. 3A). Coincident sPLA$_2$-IIA with TNF-$\alpha$ resulted in a dose-dependent enhancement of TNF-$\alpha$-stimulated PGE$_2$ production with a mean 3-fold augmentation of the TNF-$\alpha$ response over the five RSF cultures at 10 \muM sPLA$_2$-IIA (Fig. 3A). The response was significant at both 1 and 10 \muM sPLA$_2$-IIA, even though only four of the five RSF cultures were responsive.
Although sPLA₂-IIA alone had no effect on PGE₂ production in RSFs (Fig. 3A), sPLA₂-IIA (5 μg/ml) consistently increased COX-2 protein to a level similar to that induced by TNF-α (10 ng/ml) (Fig. 3, B–D). Coaddition of sPLA₂-IIA with TNF-α resulted in a synergistic increase in COX-2 protein levels (Fig. 3, B–D). The increase over sPLA₂-IIA alone was clearly detectable at 0.1 ng/ml TNF-α (Fig. 3D). COX-1 protein was not affected by these treatments, whereas a small increase in cPLA₂-α protein levels was consistently observed with TNF-α (>0.1 ng/ml)/sPLA₂-IIA treatment (Fig. 3, B and D). sPLA₂-IIA did not increase TNF-α-stimulated ICAM-1 protein expression (data not shown). Although sPLA₂-IIA also showed augmentation of IL-1β-stimulated PGE₂ production and COX-2 induction (data not shown), the response was small relative to sPLA₂-IIA augmentation of the TNF-α-stimulated response and did not occur consistently in RSF cultures.

To determine whether the responsiveness of RSFs to sPLA₂-IIA was a general feature of human fibroblasts, comparable experiments were performed using a human NLF cell line. In NLF cells, PGE₂ production was significantly but weakly increased by TNF-α (10 ng/ml) from 71 ± 3 pg/ml (n = 4) to 90 ± 10 pg/ml (n = 2) (p < 0.05). However, the weak induction was not due to a lack of responsiveness of the pathway to stimulation because IL-1β (0.1 ng/ml) stimulated basal PGE₂ production by 12-fold (p < 0.001) (Student’s unpaired t test). Also, COX-1, COX-2, cPLA₂-α, and ICAM-1 expression following TNF-α or IL-1β stimulation was similar to that observed in RSFs (data not shown). NS-398 (1 μM) abrogated TNF-α-stimulated PGE₂ production, whereas LY311727 (10 μM) had no effect. sPLA₂-IIA (10 μg/ml) did not augment TNF-α-stimulated PGE₂ production by the NLF cell line. In NLF cultures, sPLA₂-IIA, like TNF-α, also increased COX-2 protein expression, whereas coaddition of sPLA₂-IIA and TNF-α had only an additive effect on COX-2 protein levels that was not associated with increased PGE₂ production over TNF-α alone (data not shown).

sPLA₂-IIA-augmented PGE₂ production is coupled to COX-2

RSFs were stimulated with TNF-α (10 ng/ml) and increasing concentrations of sPLA₂-IIA in the presence or absence of the sPLA₂ inhibitor LY311727 (10 μM). LY311727 reduced both PGE₂ production (Fig. 4A) and COX-2/cPLA₂-α protein (Fig. 4, B and C) to levels observed in TNF-α-stimulated RSFs. The COX-2-selective inhibitor NS-398 (1 μM) reduced PGE₂ production to basal levels (Fig. 4A) without affecting TNF-α/sPLA₂-IIA-stimulated COX-2 protein (Fig. 4, B and C). Levels of COX-1 were not affected by either treatment (Fig. 4B).

Colocalization of COX-2 and PLA₂ enzymes in RA synovium

To determine whether the expression of PLA₂ enzymes was detectable in synovial tissue cells expressing COX-2, the localization of COX-2 relative to sPLA₂-IIA and cPLA₂-α in RA synovial membrane sections was examined using immunofluorescence confocal microscopy. The population of cells expressing COX-2 was first defined by double immunofluorescence with Abs to macrophage-like cells (CD68), fibroblast-like cells (5B5), and endothelial cells (factor VIII) (data not shown). COX-2 staining intensity in synovial sections derived from five independent RA patients was consistently strongest in the macrophage-like cells. Positive staining was also observed in both synovial lining and subsynovial lining fibroblast-like cells, whereas endothelial cells were usually negative or weakly COX-2 positive. Overall, the COX-2 staining pattern observed was consistent with that described previously for RA (14) with the exception that strong COX-2-positive endothelial cell staining was not consistently seen.

RA synovium from three patients was then triple-labeled with Abs to COX-2, CD14, and sPLA₂ (Fig. 5) or COX-2, CD14, and cPLA₂-α (Fig. 6). All cells positive for COX-2 were also sPLA₂-positive (Fig. 5D), although sPLA₂-positive/COX-2-negative cells were observed. The majority of CD14-positive cells were COX-2-negative, and a subpopulation of those cells were sPLA₂-positive.

**Figure 4.** Effect of sPLA₂ and COX-2 inhibitors on sPLA₂-IIA-stimulated PGE₂ production and COX-2 induction. PGE₂ production (A) was measured in RSFs (n = 5) treated for 24 h with 0.1% DMSO and increasing concentrations of sPLA₂-IIA plus TNF-α (10 ng/ml) either alone (○), or in combination with LY311727 (10 μM) (●) or NS-398 (1 μM) (□). Unstimulated PGE₂ levels ranged from 46 ± 2 to 245 ± 10 pg/ml. **, p < 0.01; ***, p < 0.001 between LY311727-treated cells and sPLA₂-IIA/TNF-α-treated cells, Wilcoxon signed rank. COX-2, COX-1, cPLA₂-α, or β-actin levels (B and C) were measured by SDS-PAGE immunoblotting and densitometry in RSF cultures (n = 3) stimulated for 18 h with sPLA₂-IIA (5 μg/ml) plus TNF-α (10 ng/ml) and 0.1% DMSO either alone (TNF-α/sPLA₂) or in combination with LY311727 (10 μM) or NS-398 (1 μM).
FIGURE 5. Colocalization of sPLA₂ with COX-2 in RA synovium. Synovial sections from patients with RA were stained simultaneously and imaged for COX-2 (A, red), sPLA₂ (B, blue), and CD14 (C, green). The overlaid image is shown in D.  

Discussion

Though sPLA₂-IIA is significantly elevated at sites of inflammation in immune-mediated disorders, the role of secreted PLA₂ forms in PG production in primary cells relevant to human inflammatory pathology or in the clinical manifestations of inflammation in vivo is not clear. Transgenic overexpression of sPLA₂-IIA causes hyperplasia of the skin but not arthritis (25). Blocking sPLA₂-IIA activity with selective inhibitors in rat adjuvant-induced arthritis shows inconsistent results between studies, whereas inhibitors are consistently effective in blocking acute carrageenan-induced inflammation (30, 31). However, interpretation of intervention studies blocking sPLA₂ in animal models is complicated because there are at least nine mammalian forms of sPLA₂ (32–36) and the tissue distribution and regulation of expression of these forms is different in rodents relative to humans. We show here that, unlike TNF-α alone, sPLA₂-IIA alone has no effect on PGE₂ production in RSFs, although it consistently up-regulated COX-2 protein to levels comparable to those seen with TNF-α alone. These data suggest that COX-2 up-regulation is necessary but insufficient for PGE₂ production. Additional signals are provided by TNF-α stimulation, which sPLA₂-IIA alone cannot provide. It is possible that sPLA₂-IIA, although enzymatically active, can neither supply substrate directly to COX-2 nor indirectly activate cPLA₂-α. TNF-α signaling likely results in post-translational activation of cPLA₂-α at the level of phosphorylation and/or mobilization to membranes. Alternatively, TNF-α may also regulate the recently cloned PGE synthase (37) immediately distal to COX-2 in the PGE₂ pathway. Importantly, COX-2 has recently been shown to mediate effects on cellular proliferation independently of its enzyme activity (38). Thus, sPLA₂-IIA, via induction of COX-2 protein, may have broader effects on cell function than modulation of PG synthesis alone.

sPLA₂-IIA amplifies TNF-α-induced PGE₂ production by RSFs at concentrations that are found in the synovial fluid of patients with RA (26). Importantly, this amplification occurs over a range of TNF-α concentrations, suggesting that expression of sPLA₂-IIA in synovium may sensitize synovial cells to produce PGs at low concentrations of TNF-α, thereby contributing to the severity of the PG-mediated synovial inflammatory response. This suggestion is supported by the observation that the spontaneous arthritis resulting from transgenic overexpression of TNF-α in mice (3) is exacerbated and is earlier in onset when human sPLA₂-IIA is transgenically overexpressed in combination with TNF-α (39).

Our experiments with NLFs show that amplification of TNF-α-induced PGE₂ production by sPLA₂-IIA is not a general feature of human fibroblasts, even though a functional cytokine-inducible COX-2-dependent PGE₂ production pathway is present in these cells. Further, sPLA₂-IIA did not consistently amplify IL-1β-stimulated PGE₂ production in RSFs, suggesting that IL-1β alone may be sufficient to maximally stimulate the cPLA₂-α/COX-2 pathway.
in these cells. The relative roles of TNF-α and IL-1β in stimulating synovial inflammation is a matter of controversy. Notably, both cytokines are capable of inducing synovial inflammation, and there is data suggesting that each is dependent on the other. Also, TNF-α is important in the induction of synovial inflammation in RA, whereas IL-1β, although able to cause inflammation, is more consistently potent at mediating cartilage degradation (2). It is likely that there is significant variability in the cytokine profiles of patients with RA depending on both genetic and environmental factors. Consequently, sPLA2-IIA effects may also vary depending on the local synovial cytokine environment.

Both TNF-α-stimulated and sPLA2-IIA augmentation of TNF-α-stimulated PGE2 production by RSFs is COX-2-dependent. This finding is consistent with the effects of exogenous sPLA2 in studies in model rodent cell lines using other agonists such as nerve growth factor stimulation of rat mast cells (40, 41). In addition, in some, but not all cases where sPLA2 has been reported to augment agonist-stimulated PG production, COX-2 protein is also up-regulated (40, 41) as has been shown here. Our immunofluorescence studies show that sPLA2 is coexpressed with COX-2 in specific subpopulations of fibroblast and CD14-positive macrophages in rheumatoid synovium, supporting the relevance of our observations with synovial cells in culture to synovium. From these studies, it is likely that the selective COX-2 inhibitors now in clinical trials with synovial cells in culture to synovium. Consequently, the mechanism by which sPLA2-IIA costimulates this pathway in RSFs. However, in our hands, sPLA2-IIA does not induce phosphorylation of p38 MAPK nor does it stimulate TNF-α-mediated phosphorylation of p38 MAPK in RSFs (42). Importantly, in contrast to findings in a murine osteoblast cell line (41), the insensitivity of the TNF-α/sPLA2-IIA-mediated induction of COX-2 to NS-398 rules out an autocrine effect of PGE2 on this pathway in RSFs.

Cytokine induction of the genes encoding COX-2 and sPLA2-α is NF-κB-dependent in RSFs (12); however, sPLA2-IIA does not affect TNF-α-induced mobilization of NF-κB into the nucleus as determined by EMSAs, Western blots for nuclear p65, or IκB degradation assays (M. J. Bidgood, M. L. Taberner, and K. F. Scott, manuscript in preparation). COX-2 is also post-transcriptionally regulated via a p38-MAPK-dependent mechanism in human monocytes (43). It is known that TNF-α can signal through the p38 pathway in chondrocytes (5), and it is possible that sPLA2-IIA coinduction of COX-2 protein expression in RSFs. However, in our hands, sPLA2-IIA does not induce phosphorylation of p38 MAPK nor does it stimulate TNF-α-mediated phosphorylation of p38 MAPK in RSFs (M. J. Bidgood, M. L. Taberner, and K. F. Scott, manuscript in preparation). Consequently, the mechanism by which sPLA2-IIA amplifies COX-2 protein expression in RSFs remains to be established.

Our studies support the view that sPLA2-IIA is one factor that may amplify TNF-α-dependent pathways in rheumatoid synovium and that the level of expression of sPLA2-IIA in synovium, together with that of TNF-α, may contribute to the severity of the
PG-mediated inflammatory response. Levels of sPLA₂-IIA are also increased in several other immune-mediated conditions. It is possible that sPLA₂-IIA may be a severity factor in these conditions also. Genome scanning studies have shown that several non-HLA loci contribute to disease susceptibility and severity in humans and in animal models of immune-mediated disease. Susceptibility loci identified in several autoimmune disorders, including RA (44), cluster to discrete chromosomal regions (45), suggesting that immune-mediated diseases may have genetic features that are shared, despite their diverse clinical manifestations. One of these loci (human chromosome 1p36-ter), identified as a susceptibility locus in multiple sclerosis and Crohn’s disease (45) and later in RA (44), overlap with the chromosomal location of the genes encoding sPLA₂-IIA, -IID, -IIE, and -I, p35–36 (33, 34, 46). Thus, as suggested by our biochemical studies and by transgenic overexpression of sPLA₂-IIA with TNF-α (39), sPLA₂-IIA may be one among several candidate genes in this region in which mutations that lead to variation in expression may contribute to onset and/or severity of immune-mediated inflammatory diseases in humans.

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