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Annexin I and dexamethasone effects on phospholipase and cyclooxygenase activity in human synoviocytes

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Annexin I is a glucocorticoid-induced mediator with anti-inflammatory activity in animal models of arthritis. We studied the effects of a bioactive annexin I peptide, ac 2–26, dexamethasone (DEX), and interleukin-1β (IL-1β) on phospholipase A_2 (PLA_2) and cyclooxygenase (COX) activities and prostaglandin E_2 (PGE_2) release in cultured human fibroblast-like synoviocytes (FLS). Annexin I binding sites on human osteoarthritic (OA) FLS were detected by ligand binding flow cytometry. PLA_2 activity was measured using ^{3}H-arachidonic acid release, PGE_2 release and COX activity by ELISA, and COX2 content by flow cytometry. Annexin I binding sites were present on human OA FLS. Annexin I peptide ac 2–26 exerted a significant concentration-dependent inhibition of FLS constitutive PLA_2 activity, which was reversed by IL-1β. In contrast, DEX inhibited IL-1β-induced PLA_2 activity but not constitutive activity. DEX but not annexin I peptide inhibited IL-1β-induced PGE_2 release. COX activity and COX2 expression were significantly increased by IL-1β. Annexin I peptide demonstrated no inhibition of constitutive or IL-1β-induced COX activity. DEX exerted a concentration-dependent inhibition of IL-1β-induced but not constitutive COX activity. Uncoupling of inhibition of PLA_2 and COX by annexin I and DEX support the hypothesis that COX is rate-limiting for PGE_2 synthesis in FLS. The effect of annexin I but not DEX on constitutive PLA_2 activity suggests a glucocorticoid-independent role for annexin I in autoregulation of arachidonic acid production. The lack of effect of annexin I on cytokine-induced PGE_2 production suggests PGE_2-independent mechanisms for the anti-inflammatory effects of annexin I *in vivo*.

Key words: Annexin I, Dexamethasone, Synoviocytes, Eicosanoid generation

Introduction

Prostaglandins, such as prostaglandin E_2 (PGE_2), mediate the pain and edema associated with arthritis, leading to the widespread use of non-steroidal anti-inflammatory drugs in the treatment of arthritis. Within the arthritic synovial lesion, fibroblast-like synoviocytes (FLS) have been implicated as a primary source of PGE_2.[1] In eicosanoid generation, phospholipase A_2 (PLA_2) and cyclooxygenase (COX, or prostaglandin H synthase) have been described as important regulatory enzymes. The hydrolytic release of arachidonic acid from membrane phospholipids is initiated by PLA_2, and arachidonic acid is then catalysed by COX enzymes for the subsequent production of PGE_2. PLA_2 exists as a secretory group II isoform ($sPLA_2$) and an arachidonic acid-selective cytosolic form ($cPLA_2$).[2] Two isoforms of COX have also been identified: a constitutive (COX1) and a mitogen/growth factor-inducible (COX2) form.[3] Studies in a COX2 gene knock-out animal model demonstrate that joint inflammation still persists in the absence of this enzyme.[4] At present, the enzymatic events responsible for the enhanced production of PGE_2 in rheumatoid arthritis (RA) are not fully understood. Knowledge pertaining to which enzyme is rate-limiting in eicosanoid generation in synovium is not fully elucidated. It is known that expression of the $cPLA_2$ and COX2 are increased by interleukin-1β (IL-1β)[5,6] and that this induction is inhibited by the anti-inflammatory action of dexamethasone (DEX).[3,6,7]

Annexin I (previously known as lipocortin-1) is a 37 kDa protein which belongs to a family of at least 13 structurally related proteins that bind to anionic phospholipids in a Ca^{2+}-dependent manner.[8] Annexin I was originally defined as a glucocorticoid-inducible inhibitor of PLA_2 activity.[9] The concept that annexin I may modulate its anti-inflammatory actions via cell

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surface binding sites has been suggested from the identification of extracellular annexin I binding sites on peripheral blood leukocytes.[10] The involvement of annexin I in the regulation of joint inflammation has now been demonstrated in several rat models of arthritis, in which a range of biological actions extending beyond effects on PLA_2 activity has been suggested.[11–13] The possibility that annexin I has anti-inflammatory activity in human arthritis has not been previously explored. We sought to further define the effects of annexin I on PLA_2, COX and prostaglandin production in human FLS, and use the known PLA_2 inhibitory properties of annexin I to investigate the rate-limiting step in synoviocyte prostaglandin production.

Materials and methods

Materials

Annexin I ac 2–26 N-terminal derived peptide (acetyl-AMVSEFLKQAWFIENEEQEYVQTVK)[14] was prepared by use of solid phase step-wise synthesis and obtained from Prof. Milton Hearn (Monash University, Clayton, Australia). Purity was more than 99% as determined by high-performance liquid chromatography. Annexin I monoclonal antibody 1B was produced from hybridoma cultures kindly donated by Dr J Browning, Biogen Inc., Cambridge, USA, and purified using saturated (50%) ammonium sulfate precipitation, protein A sepharose affinity chromatography. Affi-Gel 10 was purchased from Bio Rad, Australia. RPMI 1640 solution, sodium bicarbonate 7.5% solution, penicillin (5000 IU/ml)/streptomycin (5000 μg/ml), L-glutamine and 1 × trypsin/ethylenediaminetetraacetic acid (EDTA) solution were purchased from ICN Biomedicals, USA. Human recombinant IL-1β (specific activity 5.95×10^7 U/mg), DEX, collagenase type II (specific activity > 125 U/mg), [5,6,8,9,11,12,14,15-H]arachidonic acid, bovine serum albumin (BSA) and calcium chloride were purchased from Sigma, Australia. [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (specific activity 212 Ci/mmol, 1.0 mCi/ml) was purchased from Amersham International plc, Australia. DNase I from bovine pancreas (specific activity > 2000 U/mg) and dispase I grade II (specific activity > 0.5 U/mg) were purchased from Boehringer Mannheim, Australia. COX2 monoclonal antibody and positive isotype control mAb, IgG_1, were purchased from Transduction Laboratories, USA. Normal human IgG was purchased from CSL Pty Ltd, Australia. Control mAb, IgG_{2a}, fluoroisothiocyanate (FITC)-conjugated anti-mouse IgG was purchased from Silenus, Australia. PGE_2 enzyme linked immuno assay (ELISA) kit was purchased from Neogen Corporation, USA. Ultima-gold scintillation fluid was purchased from Packard Instrument Company, Australia.

Purification of annexin I protein

Recombinant human annexin I, a generous gift from Dr Yuko Giga-Hama (Research Centre, Asahi Co. Ltd, Japan),[15] was purified by affinity gel chromatography, as described by Sakata et al.,[16] using purified monoclonal mouse anti-human annexin I antibody. To confirm purification, samples of purified annexin I dissolved in sample buffer were separated on a 12% gel using SDS-polyacrylamide gel electrophoresis techniques. A single 37 kDa protein band was detected by western blotting using a specific anti-annexin I mAb.

Isolation and culture of FLS

FLS were obtained from synovium of osteoarthritic (OA) patients (n = 8) undergoing joint replacement surgery. All patients satisfied the American College of Rheumatology criteria for the classification of OA.[17,18] FLS were isolated and cultured as described by Koch.[19,20] In brief, the synovial-lining tissue was dissected and minced into 2–3 mm pieces and rotated in 10 ml/2 g of enzyme solution containing 2.4 mg/ml dispase (grade II, 5 U/mg), 1 mg/ml collagenase (type II) and 1 mg/ml DNase (type I) in Ca^{2+} and Mg^{2+} free Hank's balanced saline solution. FLS were cultured in 10 cm culture plates in RPMI/10% foetal calf serum at 37°C in a 5% CO_2 humidified incubator. Cells at 3rd passage were more than 99% FLS, as demonstrated by dendritic, spindle morphology, and negative staining for the pan-leukocyte antigen CD45 by flow cytometry. Cells were used between passages 4 and 9. For all experiments, FLS were seeded at 1×10^5 cells per well in 24-well culture plates in RPMI/10% FCS and allowed to adhere overnight, prior to medium being replaced with RPMI/0.1% BSA for experimental purposes. This study was approved by the institutional ethics board.

Detection of annexin I binding sites

FLS were examined using a method that detects specific saturable annexin I binding sites, as described by Perretti *et al.*[21] Briefly, surface bound annexin I was removed by washing with 1 mM EDTA/PBS. FLS were then washed with 0.1% BSA/phosphate buffered saline (PBS)/1.3 mM $CaCl_2$ and resuspended in 20 μl 0.2% BSA/RPMI at 4°C and incubated with 0–10 μM of human recombinant annexin I. Annexin I binding sites were detected by sequential incubation with 60 μg/ml annexin I monoclonal antibody 1B or matched isotype control mAb, IgG_{2a} and FITC-conjugated anti-mouse IgG. Fluorescence was analysed on a MO-FLO flow cytometer (Cytomation, Ft Collins, CO). Cells were gated according to forward and right angle scatter, using 10 000 cells for each determination. Each determination was performed in duplicate, with

annexin I binding represented by mean fluorescence intensity.

Assessment of PLA_2 activity

PLA_2 activity in FLS was determined according to Croxtall *et al.*[22] Briefly, FLS were incubated for 18 h with 1 μCi/ml [^{3}H]arachidonic acid in 0.1% BSA/RPMI. Cells were treated with 0–100 μg/ml annexin I peptide, 0, 0.1 ng/ml human recombinant IL-1β, 10^{-9}–10^{-7} M DEX for 8 h. Triplicate cultures were used for each determination. Radioactivity in the supernatant was determined using a Wallac 1409 liquid scintillation counter (Pharmacia, Finland), with results expressed as tritiated arachidonic acid released, or as a percentage of the mean result of unstimulated cells.

Determination of PGE_2 levels

PGE_2 release was determined by assaying treated supernatants, using ELISA. Each determination was performed in duplicate, with results expressed as a percentage of the mean result of the unstimulated cells. The detection limit for this assay was <0.1 ng/ml.

Assessment of COX activity

COX activity was measured according to Wilborn *et al.*[7] In brief, FLS were incubated for 30 min with 10 μM exogenous arachidonic acid. Supernatants were aspirated and assayed for PGE_2 content using ELISA. Duplicate cultures were used for each determination. COX activity was expressed as a percentage of the mean result of the unstimulated cells.

Determination of intracellular COX2

Intracellular expression of COX2 in FLS was determined using permeabilization flow cytometry, as described by Morand *et al.*[23] Briefly, cells were fixed by suspension in 2% paraformaldehyde/PBS, followed by permeabilization using 0.2% saponin/PBS. FLS were then sequentially incubated with 20 μg/ml COX2 mAb (or isotype-matched mAb control, IgG_1) and FITC-conjugated anti-mouse IgG. Permeabilization of the cells was reversed with PBS. Labelled FLS were analysed using flow cytometry. 10 000 cells were used for each determination. Intracellular COX2 protein was expressed as mean fluorescence intensity after subtraction of mean fluorescence intensity obtained with negative control mAb.

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using the Student's *t*-test, with values of $p<0.05$ regarded as statistically significant.

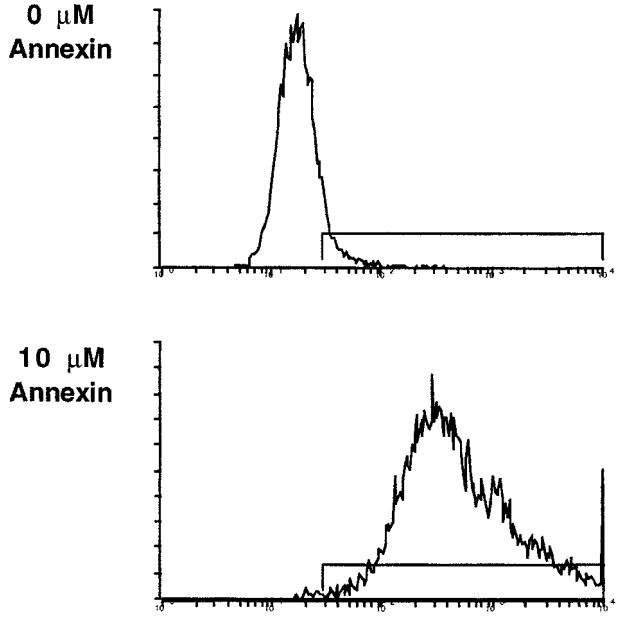

FIG. 1. Fluorescence histograms of annexin I binding sites on human FLS. FLS were incubated with rh annexin I, and annexin I binding detected by flow cytometry after labelling with an anti-annexin I mAb. Representative data obtained using (a) 0 μM and (b) 10 μM annexin I are depicted. The *x*-axis represents mean fluorescence intensity, and the *y*-axis the relative cell number. Mean data for 6 experiments are provided in Table 1.

Results

We initially sought to determine the presence of annexin I binding sites on human FLS. Annexin I binding sites as determined by mean fluorescence intensity were detected using a concentration range of annexin I (0–10 μM). Results demonstrated concentration-dependent annexin I binding, with more than 99% of cells demonstrating annexin I binding at 10 μM (Fig. 1, Table 1).

To define the effect of annexin I on synoviocyte PLA_2 activity, FLS were treated with annexin I N-terminal peptide ac 2–26 (0–100 μg/ml). Annexin I peptide induced a significant concentration-dependent inhibition of constitutive PLA_2 activity (Fig. 2(a)).

Table 1. Annexin I binding sites on human FLS

Annexin I concentration (μM)	Annexin I binding (%)	*p* value
0	3.7 ± 0.5	
1	76.7 ± 4.2	< 0.01
5	97.0 ± 0.4	< 0.01
10	99.5 ± 0.1	< 0.01

Annexin I binding on FLS was detected using a concentration range of annexin I (0–10 μM) and quantitated by flow cytometry. Values are the percentage of cells expressing fluorescence greater than that obtained with a negative control mAb, and are the mean ± SEM of 6 experiments. All concentrations of annexin I exhibited significant binding ($p<0.01$) compared to cells incubated without annexin I.

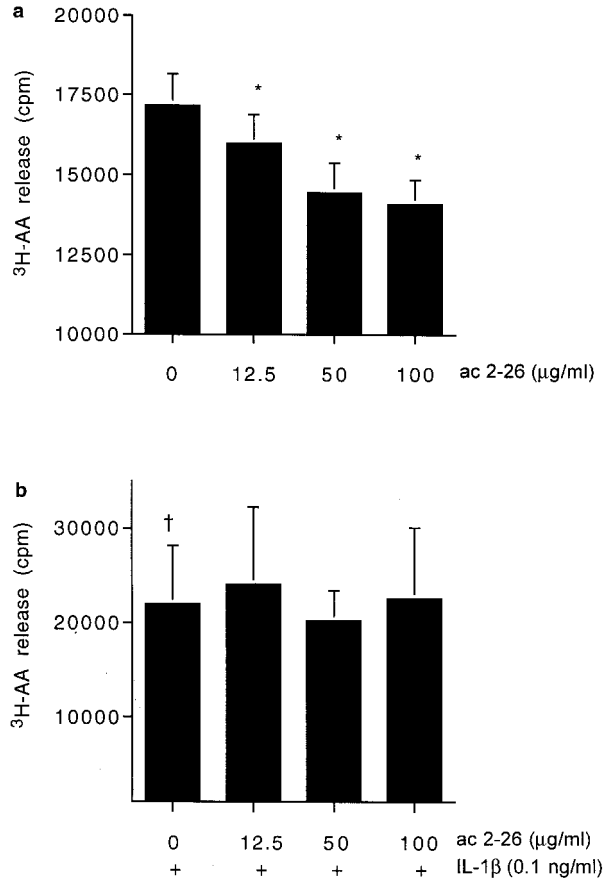

FIG. 2. Effect of annexin I peptide on human FLS PLA_2 activity. FLS were treated with (a) annexin I ac 2–26 (0–100 μg/ml), and/or (b) IL-1β (0.1 ng/ml) plus annexin I peptide for 8 h. PLA_2 activity is expressed as ^{3}H-AA released. Values are the mean ±SEM of 8 experiments. *$p < 0.05$ versus untreated FLS, $p < 0.01$ versus untreated FLS.

Inhibition of PLA_2 activity by annexin I was reversed when cells were co-stimulated with IL-1β (Fig. 2(b)).

We subsequently assessed the effects of DEX on synoviocyte PLA_2 activity. DEX treatment did not significantly inhibit constitutive PLA_2 activity over the concentration range 10^{-9}–10^{-7} M. In contrast, DEX exerted a significant concentration-dependent reduction of PLA_2 activity in IL-1β-stimulated FLS (Fig. 3).

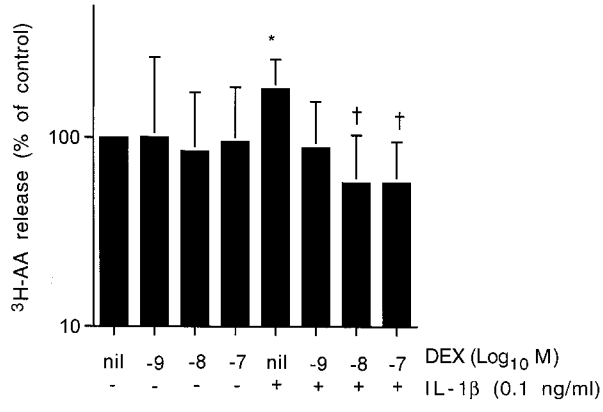

FIG. 3. Effect of DEX on human FLS PLA_2 activity. FLS were treated with DEX (10^{-9}–10^{-7} M), and/or IL-1β (0.1 ng/ml) for 8 h. PLA_2 activity (^{3}H-AA released) is expressed as a percentage of untreated FLS. Values are the mean ± SEM of 8 experiments. *$p < 0.01$ versus untreated FLS, $p < 0.05$ versus IL-1β-treated FLS.

Since annexin I peptide inhibited constitutive PLA_2 activity, we next determined whether treatment with annexin I peptide affected PGE_2 release. Constitutive PGE_2 synthesis was detected in FLS. Annexin I peptide did not significantly reduce constitutive PGE_2 synthesis (Fig. 4(a)). A significant increase in PGE_2 release was observed following IL-1β stimulation (p=0.001), and annexin I peptide did not inhibit IL-1β-stimulated PGE_2 release. In contrast, DEX had no effect on constitutive PGE_2 release but significantly inhibited IL-1β-induced PGE_2 release in a concentration-dependent manner (Fig. 4(b)).

To further address the differential inhibitory actions of annexin I on PLA_2 activity and PGE_2 synthesis, studies were designed to evaluate the effects of annexin I peptide and DEX on constitutive and IL-1β-induced synoviocyte COX activity. Since the synthesis of PGE_2 from endogenous arachidonic acid results

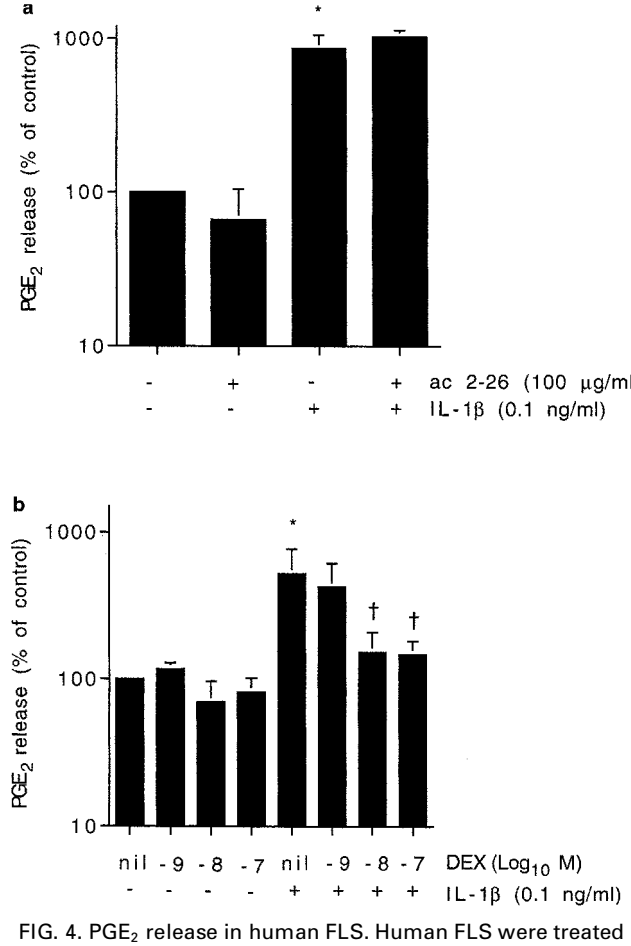

FIG. 4. PGE_2 release in human FLS. Human FLS were treated with IL-1β (0.1 ng/ml), and/or (a) annexin I ac 2–26 (100 μg/ml), (b) DEX (10^{-9}–10^{-7} M) for 8 h. PGE_2 release was quantitated by assaying supernatants using ELISA and expressed as a percentage relative to untreated FLS. Values are the mean ±SEM of 4 experiments. *$p < 0.01$ versus untreated FLS, $p < 0.05$ versus IL-1β-treated FLS.

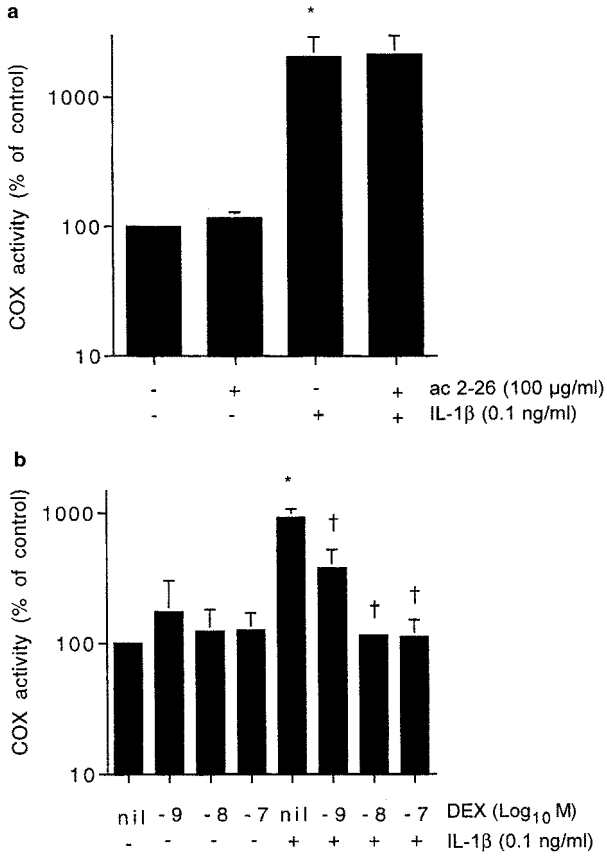

FIG. 5. COX activity in human FLS. FLS were treated with IL-1β (0.1 ng/ml), and/or (a) annexin I ac 2–26 (100 µg/ml), (b) DEX (10^{-9}–10^{-7} M) for 8 h and COX activity measured as described. COX activity is expressed as a percentage relative to untreated FLS. Values are the mean ± SEM of 4 experiments. *$p < 0.01$ versus untreated FLS, $p < 0.05$ versus IL-1β-treated FLS.

from the activities of both PLA_2 and COX, to estimate maximal COX activity, PLA_2 activity was bypassed by addition of exogenous arachidonic acid.[7] Low levels of constitutive COX activity were detected in FLS. IL-1β significantly increased COX activity (p=0.0004) (Fig. 5(a)). Annexin I peptide exerted no significant effect on constitutive or IL-1β-stimulated COX activity (Fig. 5(a)). DEX exerted no significant effect on constitutive COX activity in DEX treated FLS. In contrast, DEX resulted in a concentration-dependent reduction of IL-1β-induced COX activity (Fig. 5(b)).

The question of whether modulation of COX activity reflected its intracellular expression was investigated using permeabilization flow cytometry. Constitutive expression of intracellular COX2 protein was detected in FLS, and was increased by IL-1β. Annexin I peptide did not demonstrate any significant effect on constitutive or IL-1β-stimulated COX2 protein in FLS (Fig. 6(a)). DEX did not significantly inhibit constitutive intracellular expression of COX2. However, DEX exerted a concentration-dependent inhibition of intracellular COX2 expression in IL-1β-stimulated FLS (Fig. 6(b)). These results demonstrate consistency between modulation of intracellular expression of COX2 and its activity.

Discussion

The hypothesis that annexin I is an anti-inflammatory mediator in arthritis originated from the detection of annexin I in human rheumatoid synovium.[24] Animal models of arthritis have subsequently demonstrated that annexin has important inhibitory effects *in vivo*. For example, administration of annexin I peptide substantially inhibited carrageenan-induced arthritis, whilst anti-annexin I antibody exacerbated arthritis severity and reversed the effect of exogenous DEX in this model.[12] Similarly, exacerbation of disease and increased synovial production of tumour necrosis factor-α and PGE_2 were observed in adjuvant arthritic rats administered anti-annexin I antibody.[13] Moreover, annexin I neutralization reversed the effects of DEX on disease severity in adjuvant arthritis[13] and on rat synovial macrophage nitric oxide production.[11] In human disease, histological studies demonstrate

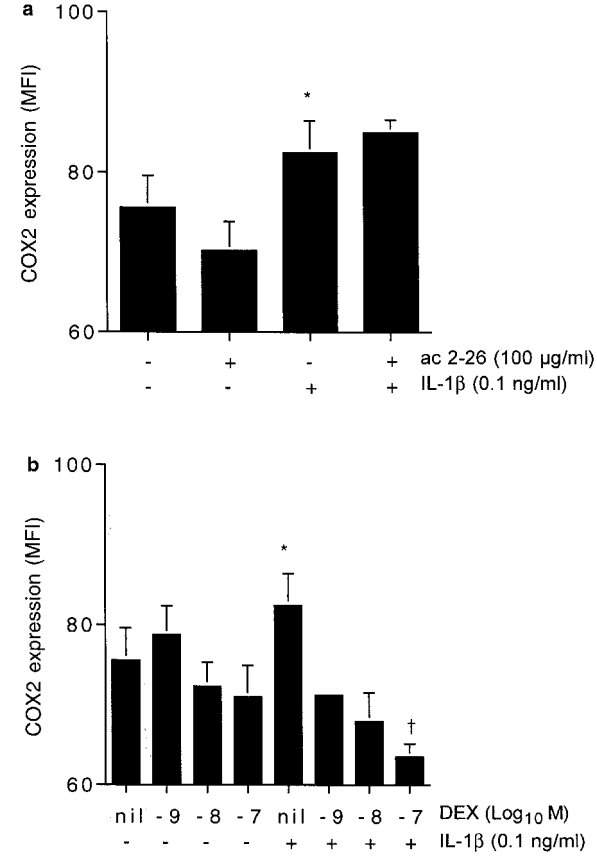

FIG. 6. Intracellular COX2 expression in human FLS. Human FLS were treated with IL-1β (0.1 ng/ml), and/or (a) annexin I ac 2–26 (100 µg/ml), (b) DEX (10^{-9}–10^{-7} M) for 8 h. Intracellular COX2 was detected by permeabilization flow cytometry and expressed as mean fluorescence intensity (MFI) after subtraction of MFI obtained with the negative control mAb. Values are mean ± SEM of 4 experiments. *$p < 0.01$ versus untreated FLS, $p < 0.01$ versus IL-1β-treated FLS.

annexin I in the synovial-lining layer of RA synovium.[24] Annexin I expression in human peripheral blood leukocytes has been described, with maximal annexin I content in neutrophils and monocytes.[23] Moreover, altered annexin I biology is seen in subjects with RA, who exhibit reduced numbers of annexin I binding sites on leukocytes compared to controls.[25] Impaired glucocorticoid induction of annexin I production has also been noted in leukocytes of RA patients,[26] and RA patients with high titre anti-annexin I antibodies exhibit impaired glucocorticoid responsiveness.[27]

Treatment of undifferentiated cells with glucocorticoids increases annexin I transport to the cell surface.[28] The mechanisms whereby annexin I crosses the membrane are unclear since the protein lacks a signal sequence and is therefore unlikely to access secretory vesicles for release via the conventional method of exocytosis. The translocation of annexin I protein from intracellular stores to cell surface binding sites is believed to be imperative for its function.[29,30] Certainly, extracellular anti-annexin I antibodies significantly reverse the anti-inflammatory effects of glucocorticoids and simultaneously deplete intracellular cellular annexin I.[11,13,31,32]

Evidence that exogenous annexin I modulates its biological effects through specific cell surface annexin I binding sites originated from the identification of these sites on peripheral blood leukocytes.[10] Given that annexin I is expressed in RA synovium, it is conceivable that in FLS, annexin I mediates its biological effects via binding to cell surface sites. No previous study has examined the presence of binding sites or the biological function of annexin I in human synovial cells.

The current data establish the presence of annexin I binding sites on human FLS. The factors influencing annexin I binding site expression are unclear, and the absence of molecular identification of the binding site limits study of its regulation. Nonetheless, having established the presence of potentially biologically active annexin I binding sites in FLS, we sought to assess the effect of annexin I on these cells. An annexin I N-terminal peptide of 25 amino acids, annexin I ac 2–26, has been shown to mimic anti-inflammatory actions of annexin I,[33,34] including inhibition of arthritis models.[11] Perretti *et al.* have reported that the biological activity of annexin I ac 2–26 is comparable with that of recombinant human annexin I, albeit at a lower molar potency, and that it requires binding to cell surface binding sites to exert its biological effects.[34]

Previous investigations have evaluated the effects of glucocorticoids and cytokines on the regulation of PLA_2 and COX at the levels of transcription[6,35] or translation.[36] Studies have also compared the level of activity of these enzymes in various cell types, following IL-1[37] and DEX treatments,[7] but studies of the rate-limiting step at the enzyme activity level in human FLS have not been undertaken. Our results indicate a significant concentration-dependent inhibition of constitutive but not IL-Iβ-induced PLA_2 activity by annexin I ac 2–26. In A549 cells, inhibition of arachidonic acid release by annexin I peptide is believed to be mediated through inhibition of the activation of $cPLA_2$ whereby $cPLA_2$ is not phosphorylated in the presence of annexin I.[38] Details of how this is accomplished are still uncertain, but binding site-dependent effects of ac 2–26 are unlikely to relate to the previously proposed 'substrate binding' hypothesis for the effects of annexin I on PLA_2. The selective inhibitory action of DEX on IL-1β-stimulated but not constitutive $cPLA_2$ transcription and expression has been described in other investigations[6,35] but is confirmed at the enzyme activity level for the first time in the current study. The uncoupling of the effects of annexin I and glucocorticoids on PLA_2 supports the contention that the constitutive anti-inflammatory effects of annexin I are only in part related to mediating the effects of glucocorticoids.

As previously demonstrated, constitutive PGE_2 synthesis was detected in cultured FLS. Cytokine upregulation of PGE_2 synthesis was observed, again consistent with previous findings.[6,36,37] Annexin I peptide did not demonstrate any significant inhibitory effect on constitutive or IL-1-stimulated PGE_2 production. In rat astrocytes, annexin I peptide reduced but did not abolish endotoxin-induced PGE_2 release.[38] Consistent with its effects on PLA_2 activity, DEX significantly reduced IL-1β-stimulated but not constitutive PGE_2 release.[7,37,38]

Annexin I mAb, however, has been observed to reverse the effects of DEX inhibition on PGE_2 release, and this study also suggested that glucocorticoid suppression of COX occurs via an annexin I-independent mechanism.[39]

Glucocorticoid-induced suppression of COX2 appears to be independent of annexin I and is almost certainly explained by the direct interaction of the steroid-receptor complex with nuclear factor-kappa B.[40] DEX inhibition of cytokine- or mitogen-induced COX regulation has been previously reported.[6,7,41,42] In this study, regulation of intracellular COX2 expression was found to be consistent with regulation of its activity, suggesting that the level of COX activity is dependent upon intracellular levels of COX2.[43] The differential effects of annexin I peptide and DEX on PLA_2 activity, COX, and PGE_2 production support the hypothesis that COX is the rate-limiting enzyme in IL-1β-induced PGE_2 synthesis in FLS. In contrast, the lack of effect of glucocorticoids on constitutive PLA_2 and COX2 activity suggests that annexin I may have a glucocorticoid-independent role in constitutive regulation of PLA_2 activity. Biological activity of annexin I peptide has been demonstrated *in vivo* in rat carrageenan arthri-

tis,[12] a model mediated by eicosanoids in addition to nitric oxide and reactive oxygen species. That annexin I peptide has previously been demonstrated to have inhibitory effects on nitric oxide and reactive oxygen species production in other systems highlights the importance of viewing annexin I not solely as an inhibitor of PLA_2 activity.

In summary, three novel findings are reported in this study. Firstly, the presence of annexin I binding sites on synoviocytes is demonstrated. Secondly, uncoupling of the effects of annexin I and glucocorticoids on constitutive and cytokine-induced arachidonic acid generation suggests that annexin I has glucocorticoid-independent regulatory activities in inflammation. Our results are consistent with the conclusion that COX2 is the rate-limiting step in synoviocyte PGE_2 synthesis, suggesting that PLA_2-directed strategies may not successfully inhibit eicosanoid production in arthritis. Thirdly, the lack of effect of annexin I on PGE_2 synthesis, despite clear effects on inflammation *in vivo*, suggests the anti-inflammatory effects of annexin I are eicosanoid-independent. For example, annexin I may influence arachidonic acid-mediated intracellular signal transduction. Arachidonic acid influences activation of the Jun-N terminal kinase/stress activated protein kinase subgroups of the membrane activated protein kinase family of signal transduction enzymes, via an eicosanoid-independent pathway.[44] This important pro-inflammatory signal pathway is also influenced by reactive oxygen species,[45] also known to be inhibited by annexin I.[46] The possibility that annexin I modulates inflammation via eicosanoid-independent mechanisms remains to be further investigated. Studies directed at understanding the role of annexin I in such pathways may have therapeutic benefit in the treatment of arthritis.

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