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RECOMBINANT TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 BOTH STIMULATE HUMAN SYNOVIAL CELL ARACHIDONIC ACID RELEASE AND PHOSPHOLIPID METABOLISM

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Summary Stimulation of [^{3}H]-arachidonic acid labeled human synovial cells with 3.0×10^{-10}M recombinant interleukin-1 or tumor necrosis factor resulted in the release of incorporated radiolabel (41.1% and 27.7% respectively). Analysis of [^{3}H]-arachidonic acid labeled phospholipids showed that interleukin-1 and tumor necrosis factor diminished [^{3}H]-arachidonic acid in phosphatidylcholine phosphatidylserine and phosphatidylinositol. Treatment of [^{3}H]-arachidonic acid labeled cells with 10^{-3}M dibutyryl cyclic AMP or 10^{-6}M PGE_2 did not affect spontaneous or stimulated [^{3}H] arachidonic acid release significantly. These data show that recombinant interleukin-1 and tumor necrosis factor stimulate human synovial cell phospholipase activity in a similar manner and that this activity is not affected significantly by agents that elevate cyclic AMP.

Inflammatory diseases are characterized by edema, cellular infiltration, tissue destruction and release of arachidonic acid metabolites (1). An increasing amount of evidence has shown that the cytokines IL-1 and TNF mediate mechanisms involved in inflammation and tissue damage. These cytokines share the ability to induce neutrophil degranulation (2,3), bone resorption (4,5), PGE_2 production (6,7), procoagulant activity (8), and collagenase secretion (6,7). PGE_2 production is dependent upon prior release of arachidonic acid by a phospholipase(s) (9), and it has been demonstrated that phospholipase A_2 can be activated by IL-1 in rabbit chondrocytes (10). Induction of PGE_2 pro-

Abbreviations: rIL-1; recombinant human interleukin-1 beta, rTNF; recombinant human tumor necrosis factor alpha, cAMP; adenosine 3',5'-cyclic monophosphate, d-cAMP; dibutyryl cyclic AMP, AA; arachidonic acid, PGE_2; prostaglandin E_2, PC; phosphatidylcholine, PS; phosphatidylserine, PI; phosphatidylinositol, PE; phosphatidylethanolamine, PLA_2; phospholipase A_2, PLC; phospholipase C, DPBS; Dulbecco's phosphate buffered saline, DMEM + 10% FBS; Dulbecco's modified Eagle medium plus 10% fetal bovine serum, LPS; lipopolysaccharide.

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duction in macrophages and synovial cells by IL-1 or TNF (6,7,11) suggests that these cytokines may activate phospholipase activity in these cells as well. In return, PGE_2 which activates adenylate cyclases, inhibits IL-1 and TNF-induced responses (11,12), and has been proposed as a possible autoregulatory mechanism during IL-1 stimulation (13).

In the present report we examine recombinant IL-1 and TNF-induced arachidonic acid release and phospholipid metabolism in human synovial cells, and the effects on these parameters of cAMP elevating agents.

MATERIALS AND METHODS

Reagents and Chemicals Solvents were obtained from Fisher Scientific (Springfield, NJ) or Thomas Scientific (Swedesboro, NJ), radiochemicals from New England Nuclear (Boston, MA), tissue culture media from GIBCO (Grand Island, NY) and all other reagents were obtained from the Sigma Chemical Co. (St. Louis, MO). Human rIL-1 beta and human rTNF alpha were cloned, expressed, and purified by the Departments of Molecular Genetics and Protein Biochemistry and Macromolecular Science at Smith Kline & French Laboratories. rIL-1 beta was cloned and expressed from a human peripheral blood monocyte cDNA library essentially as described by Auron et al. (14). rTNF alpha was cloned and expressed using the TNF sequence as described by Pennica et al. (15). The physicochemical and biological properties of rIL-1 are comparable to natural IL-1 (16), while rTNF has properties similar to those described for rTNF (M.-J. Chen, unpublished data). Specific activity both cytokines was assessed at 3.0×10^8 units/mg using the method of Simon et al. (17) for IL-1 and a modification of a previously described method (18) for TNF. The LPS content of the cytokines was less than 0.2 ng/ 100ug of recombinant material.

Cell Preparation and $[^3H]$-AA Labeling Synovium from the knee of an osteoarthritic patient was obtained from Dr. Bruce Smith, Thomas Jefferson Medical College, Philadelphia and the tissue was processed as described by Dayer et al. (19). Confluent monolayers were trypsinized and 1 mL (about 5×10^5 cells) aliquots were placed in 24 well tissue culture dishes (Costar, Cambridge, MA) containing 12 mm glass coverslips (Fisher Scientific). $[^3H]$-AA (83.8 Ci/mmol, 1.2 uCi/$5x10^5$ cells) was added to the dishes and incubated for 15-18 hours. After this time the cells were washed 3 times in DPBS and treated with DMEM-10% FBS alone or containing the appropriate treatment for the experimental time period. The cells used for experiments were between passage level four and eight.

Assay For $[^3H]$-AA Labeled Phospholipids and $[^3H]$-AA Release After the completion of the experiment, the supernatants were removed, combined with scintillation cocktail (Beckman Hp/b) and counted in a scintillation counter (Beckman LS 7800). The coverslips were then removed and extracted with successive 300 ul aliquots of chloroform/methanol (2:1), chloroform, and 4M potassium chloride (each containing 0.08% butylated hydroxytoluene). After overnight incubation (4°C), a 100 ul aliquot of the organic phase was spotted on a Silica Gel G thin layer chromatography (TLC) plate (Analtech, Newark, DE) under a constant flow of nitrogen. The TLC plates were then run using a previously described method (20) and radiolabel quantitated by the Bioscan Imaging Scanner 200 (Bioscan, Washington, DC).

RESULTS

Time Course and Dose Response of TNF and IL-1 induced $[^3H]$-AA Release rTNF and rIL-1 treatment increased the rate of $[^3H]$-AA release within 30 min of

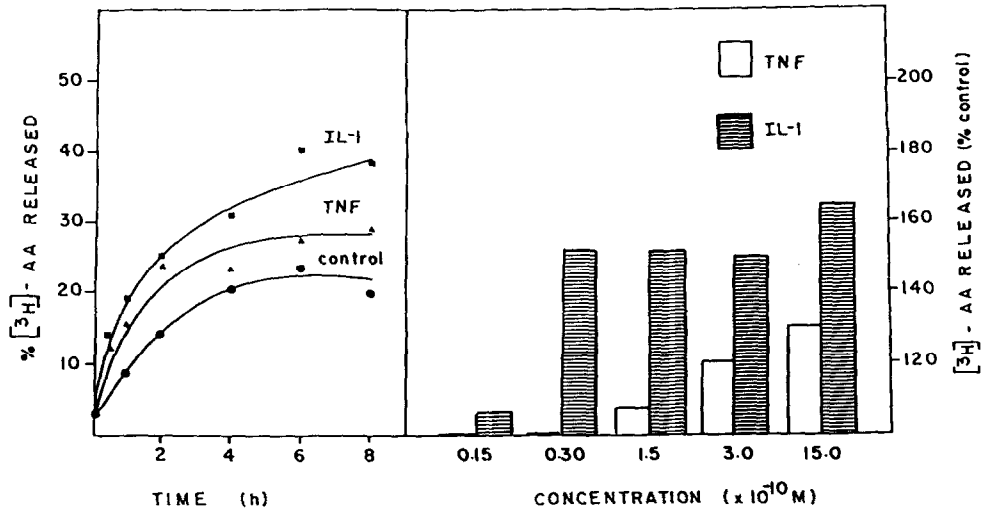

Figure 1. Time course [A] and dose response [B] of [^{3}H]-AA release from human synovial cells. Data is expressed as a percentage of the total incorporated [^{3}H]-AA (100% = 8.04 x 10^5 dpm) [A] or by the % increase over the control value [B]. The results are expressed as the mean of duplicate determinations.

exposure (0.33 and 0.37%/min respectively) as compared to the control (0.12%/min, Figure 1A). The rTNF-induced rate of release returned to control levels by 6h while the IL-1 stimulated rate was still elevated. The data in Figure 1 (B) also shows that maximal rates of release at 5h were achieved with 15x10^{-10}M of either cytokine. The following experiments were performed using 3.0 x 10^{-10}M for a 5h time period.

Distribution of [^{3}H]-AA Label After Stimulation At five hours post-stimulation, cellular lipids were extracted and the distribution of [^{3}H]-AA labeled phospholipids assessed (Table 1.). A decline in [^{3}H]-AA in PC, PI, PE, and neutral lipids occurred during the first 5h in control cells, correlating with the spontaneous release observed. rTNF (3x10^{-10}M) treated cells had an additional decline in [^{3}H]-AA label from PC, PS, and PI. This effect was also seen with 3x10^{-10}M of IL-1; however, the decline elicited by this cytokine was greater.

Effect of d-cAMP and PGE_2 on [^{3}H]-AA Release [^{3}H]-AA release from unstimulated and stimulated synovial cells was not inhibitable with 10^{-6}M

Table 1. Effect of rIL-1 and rTNF on the Cellular Distribution of [^{3}H]-AA in Human Synovial Cells

Treatment	Time (h)	% of total incorporated [3H]-AA					
		PC	PS	PI	PE	NL[1]	AA (rel)
Medium	0	33.8	7.9	20.1	15.3	23.6	0.9
Medium	5	27.6	7.4	13.3	16.3	19.7	15.4
rTNF ($3x10^{-10}$M)	5	22.9*	6.1*	9.6*	17.4	19.6	21.6
rIL-1 ($3x10^{-10}$M)	5	16.0**	4.7**	7.9**	17.3	17.9	36.6

Results expressed as the mean of 3 to 5 experiments, *rTNF (5h) vs control (5h) PC - $p< 0.025$, PS - $p< 0.05$, PI - $p< 0.005$; **rIL-1 (5h) vs control (5h) PC, PS, PI - $p< 0.0005$. Statistical analysis on data performed using Students one tailed t test.

[1] Neutral lipids and trace amounts unidentified radiolabeled substances

PGE_2 (Figure 2.). Pretreatment with 10^{-3}M d-cAMP induced a slight inhibition from control cells and the cells stimulated with $3x10^{-10}$M rTNF or rIL-1 but this inhibition was of marginal statistical significance ($p > 0.05$).

DISCUSSION

IL-1 and TNF induced cellular responses that mimic the inflammatory reactions presumed to occur in rheumatoid arthritis. In our experiments 3.0×10^{-10}M IL-1 or TNF enhanced the release of [^{3}H]-AA from prelabeled human synovial cells in a time and dose dependent fashion. Although the released radiolabel was not identified by us, others have demonstrated increased generation of PGE_2 by IL-1 or TNF stimulated human synovial cells (6,7,11). The rate of [^{3}H]-AA release (Figure 1A) between 30 min and 2h post-stimulation was similar with both cytokines; however, TNF-mediated release (unlike IL-1) dropped to control levels by 6h.

Liberation of arachidonic acid from membrane phospholipids requires the activation of specific phospholipases (21). PLA_2 primarily utilizes PE, PC,

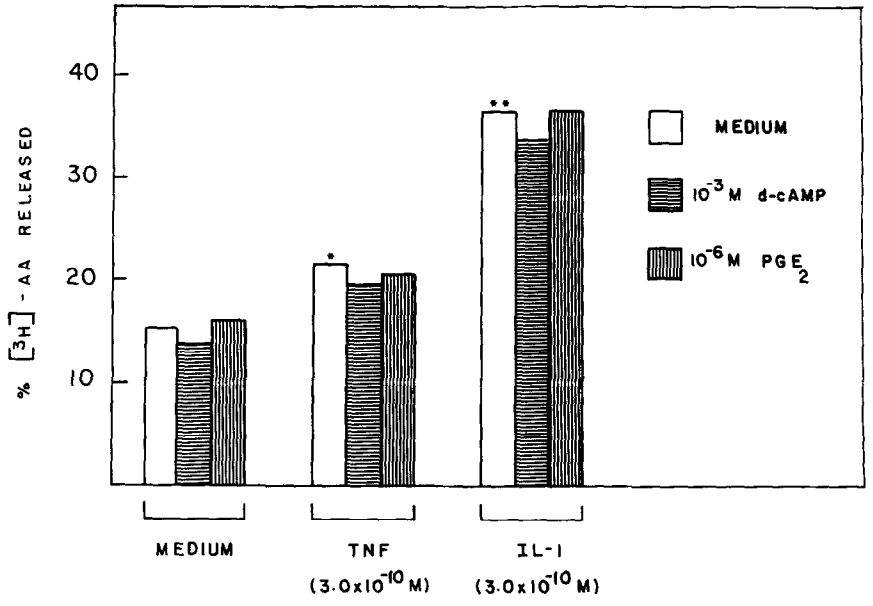

Figure 2. Effect of d-cAMP or PGE_2 on [^{3}H]-AA release from human synovial cells. [^{3}H]-AA release is expressed as a percentage of the total incorporated [^{3}H]-AA and represent the mean of 4 experiments, *$p < 0.0005$ vs control (5h), ** $p < 0.0001$ vs control (5h). (100% = $1.2 \times 10^6 \pm 3.4 \times 10^5$ dpm, mean $\pm$ SD).

and PS as substrates while PI is utilized by PLC (21). Chang et al. (10) have shown that PLA_2 is increased when rabbit chondrocytes are stimulated by IL-1. In our study [^{3}H]-AA labeled cellular phospholipids were examined to determine the phospholipid pool (and thus the potential phospholipase) activated during cytokine stimulation of human synovial cells. Challenge with IL-1 or TNF decreased [^{3}H]-AA label from PC, PS, and PI, suggesting the enhancement of PLA_2 and PLC activities. On the other hand, human synovial cell phospholipase activities in the have not been fully characterized and a PLC or PLA_2 exhibiting alternate substrate specificities may be present.

Activation of phospholipase activity in platelets and macrophages is substantially suppressed by prostaglandins and other cyclic AMP elevating agents (22,23), possibly by controlling the availability of free calcium (24). Because IL-1-induced plasminogen activator secretion and TNF-induced IL-1 stimulation are substantially inhibited by PGE_2 (11,12), the possibility existed that PGE_2 induced increases in cAMP might be involved in regulation

of IL-1 and TNF-induced responses. Our findings show that treatment of synovial cells with cyclic AMP elevating agents failed to inhibit IL-1 and TNF mediated [^{3}H]AA release (phospholipase activity) significantly.

In conclusion, our report shows the rIL-1 and TNF enhanced release of [^{3}H]-AA from human synovial cells by stimulation of a phospholipase A_2 and possibly phospholipase C. Moreover, [^{3}H]-AA release from synovial cells mediated by IL-1 or TNF is not significantly inhibited by intracellular cyclic AMP.

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