INTRODUCTION
IL-1 is elevated in arthritic joints and in mechanically stressed (1, 2) cartilage where its inhibitory actions likely contribute to cartilage loss and mitigate against effective maintenance and repair. IL-1 induces cyclooxygenase-2 (COX-2) in arthritic cartilage, causing synthesis of high concentrations of PGE_2 (3). COX-2 inhibitors are widely used to relieve the inflammation and pain associated with arthritis (4). Activated p38 mitogen-activated protein kinases (MAPK) are also implicated in the actions of IL-1. Specific inhibitors have been developed which show promise for the treatment of experimental arthritis (5) and are in clinical trials for other conditions (6). The current studies compare the ability of p38 MAPK inhibitors and COX-2 inhibition to blunt the inhibitory actions of IL-1 on arthritic human chondrocyte proliferation and proteoglycan synthesis.

METHOIDS AND MATERIALS
Chondrocytes were isolated from arthritic human femoral condyle cartilage obtained at total knee replacement under an IRB exempt protocol. The cells were grown to 80% confluence in DMEM/1% PS/10% FCS and the serum concentration reduced for 24 hr before addition of the COX-2 inhibitor Sc 58125, the p38 MAPK inhibitors Sb 203580 or Sb 202190. IL-1, and growth factors as noted in the figures. Proliferation was measured as ^3H thymidine incorporation into TCA precipitated material during a 2 hr pulse label and proteoglycan synthesis measured as ^35S sulfate incorporation during a 5-hr pulse (7). Conditioned media (CM) nitric oxide was measured as nitrite with the Griess reaction and CM PGE_2 was assayed with the ELISA kit from Amersham Pharmacia Biotech. Data is from 4-6 preparations with each value representing 6-12 wells. Statistically significant differences were determined using Student’s t-test.

RESULTS

**Figure 1.** COX-2 and p38 MAPK inhibition blunt the inhibitory effect of IL-1 on human chondrocyte proliferation.

Chondrocytes were grown to 80% confluence, medium serum reduced, and 1 uM Sb 203580 or 0.5 uM Sc 58125 added 30 minutes before IL-1. 50 pM TGF-beta, 50 ng/ml IGF-1 or 5% FCS were added after 6 hr, and proliferation assayed 24 hr later. Values are mean ± SE of n=9-12.

*p<0.05 vs. IL-1.

Both p38 MAPK (Sb) and COX-2 (Sc) inhibition increased proliferation in IL-1 activated chondrocytes under basal and growth factor stimulated conditions (Fig 1), although not to the levels seen in the absence of IL-1 (data not shown). Similar results were seen with a second p38 MAPK inhibitor, Sb 202190. Neither p38 MAPK nor COX-2 inhibition altered IL-1 activated human chondrocyte NO production [CM values were 12.4±0.44 uM (IL-1), 11.8±0.85 (IL-1+Sb), and 11.4±0.82 (IL-1+Sc)]. The p38 MAPK inhibitor Sb did however decrease CM PGE_2 from 52±5 ng/ml to 13±1.7 ng/ml in the presence of IL-1, and from 71±4 to 38±5 ng/ml in chondrocytes exposed to both IL-1 and TGF-beta. COX-2 inhibition (Sc) reduced CM PGE_2 to less than 0.5 ng/ml under both basal and IL-1 activated conditions. The sensitivity of human chondrocyte proliferation to exogenous PGE_2 within this concentration range was evaluated, and concentrations as low as 1 ng/ml caused significant inhibition (TGF-beta stimulated values were reduced by 50%; FCS stimulated proliferation was reduced by 20%). This suggests part of the effect of p38 MAPK inhibition could be secondary to the reduction in the synthesis and accumulation of PGE_2.

As seen in Figure 2, IL-1 effectively blocks the ability of TGF-beta to stimulate proteoglycan synthesis, reducing the increase above control values from 54 to 9 pmol/10 mg wet wt, an 83% decrease. p38 MAPK inhibition with Sb 202190 blunted TGF-beta stimulated proteoglycan synthesis by 37%, thus providing no relief from the anti-anabolic actions of IL-1. In contrast, COX-2 inhibition (Sc) of PGE_2 synthesis restored IL-1 inhibited proteoglycan synthesis to within 80% of control.

**Figure 2.** Inhibition of PGE_2 synthesis in IL-1 Activated Chondrocytes Restores TGF-beta Stimulated Proteoglycan Synthesis.

Chondrocytes were activated with 1 ng/ml IL-1 with or without the pre-addition of 1 uM Sb 202190 or 0.5 uM Sc 58125 added 6 hr later, and proteoglycan synthesis evaluated the following day. Data is given as TGF-beta stimulated synthesis. Values are mean ± SE of n=6-9. *p<0.05 vs. Vehicle Control; #p<0.05 vs. IL-1.

DISCUSSION
In IL-1 activated chondrocytes from arthritic human joints, inhibitors of both COX-2 and p38 MAPK potentiated growth factor stimulated DNA synthesis. These results suggest that part of IL-1 inhibition of human chondrocyte proliferation is mediated through p38 MAPK activation and increased PGE_2 synthesis. The data indicate IL-1 inhibition of matrix protein synthesis in the presence of TGF-beta may primarily be the result of the concomitant increase in PGE_2. p38 MAPK and COX-2 inhibition have unique and overlapping abilities to counteract the anti-anabolic actions of IL-1 in human chondrocytes, and as such show promise to minimize cartilage damage in arthritic and mechanically stressed joints.

REFERENCES

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