CATABOLISM OF AGGREGAN BY CELLS DERIVED FROM OSTEOARTHRITIC BONE.

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INTRODUCTION Osteoarthritis (OA) is characterized by a focal loss of cartilage, stiffening of the subchondral bone and osteophyte formation. However, it is not clear whether changes occur first in the cartilage, the bone or are concurrent. Scintigraphic scans show bony changes are an early feature of OA and predict cartilage loss (1). In addition, correlations between positive bone scans and synovial fluid markers of bone and cartilage turnover suggest interactions between the two structures (2). To determine whether bone cells could alter cartilage metabolism, we devised a co-culture system. Our results provided the first evidence that products released from OA bone derived cells could degrade cartilage, as judged by increased GAG release (3). Subsequent work suggests bone cells produce enzymes that directly degrade cartilage matrix (4). The aims of the present study are to identify the enzymatic mediators of the effects of bone cells on cartilage.

METHODS Tissue isolation and co-culture: Tissue was obtained from a 75-year-old female patient, who fulfilled the ACR criteria for OA, undergoing surgery for total knee replacement. Cartilage loss, consistent with patellofemoral disease, was apparent on the lateral anterior femoral condyle. Explants of bone were excised from specific areas on the lateral and medial condyles of the joint (3). Bone fragments were prepared (5) and cultured in 25 cm² flasks with DMEM containing 2mM L-glutamine, antibiotics and 25mM HEPES and supplemented with 10% FBS and 50U/ml penicillin/streptomycin.

RESULTS Effects of bone cells on cartilage: Cells derived from regions 1, 2 and 3 of an OA knee were examined for their ability to alter cartilage metabolism. Bone cells derived from Region 1, not 2, 4 or 5, altered GAG release from cartilage. A significant increase in GAG release from NA cartilage biopsies incubated in the presence of cells derived from Region 1, as compared with that released from biopsies incubated in medium alone (p<0.005), was apparent after 9 days of co-culture.

IMMUNOBLOT OF CO-CULTURE SUPERNATANTS: Co-culture supernatants from cartilage incubated without (control) and with bone cells from Region 1 (test) contained aggrecan catabolites generated by aggrecanase activity (Fig.1a). Differences in the amounts of BC-3 reactive aggrecanase-generated catabolites present in test, as compared with control, were apparent after 13 days of co-culture. The quantity of aggrecanase generated catabolites in test SN increased with time in culture until day 28, when smaller fragments bearing the epitope recognized by BC-3 were also detected. Co-culture supernatants from the same test and control supernatants also contained aggrecan catabolites generated by metalloproteinase activity (Fig 1b) By contrast, no difference in the amounts of BC-14 reactive MMP generated catabolites was apparent in test, as compared with control SN, and the levels of MMP generated catabolites in control and test SN diminished with time.

DISCUSSION Here we demonstrate for the first time the presence of aggrecanase-generated fragments in supernatants from OA cartilage cultured in the presence of OA bone derived cells (T) or in medium alone (C).


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