Processing of proteoglycans after uptake by THP-1 cells

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Cartilage proteoglycans (PG) can induce and act as a target in autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis and relapsing polychondritis [1-7]. Synovial fluids and cells from patients with rheumatoid arthritis contain large numbers of activated monocytes and macrophages which express class II antigens [8, 9] and are therefore capable of acting as antigen-presenting cells in immune reactions. Increased infiltration by these cells is often accompanied by large increases in lysosomal hydrolases and proteases in synovial fluids of both naturally occurring and experimental models of arthritis [10]. Macrophages also secrete a number of cytokines, including interleukin 1 and tumour necrosis factor-α (TNF-α), both of which can promote destruction of cartilage matrix and bone [11-13]. The presence of these cytokines in synovial fluid may stimulate macrophages or chondrocytes to secrete enzymes which can degrade cartilage matrix, therefore exposing epitopes which may initiate or prolong an antigen-driven arthritis. Although endocytosis and degradation of PG has been described in human fibroblasts [14] and liver endothelial cells [15, 16], little is yet known about the processing of these molecules by macrophages and the effect of such processing on their antigenicity.

We investigated processing using human articular cartilage PG, a gift from M. Bayliss, Kennedy Institute [17]. This was labelled using 2 mCi of $^{125}$I/5 mg of PG [18] and free label was removed by gel filtration. Some samples of iodinated PG aggregate were subjected to proteolysis using either trypsin (1.5 mg of PG: 3 μg of trypsin for 6 h at 37°C in 0.05 M-Tris, 0.1 M-NaCl buffer, pH 7.5) or stromelysin (1.5 mg of PG: 1 unit of stromelysin for 16 h at 37°C in 0.01 M-CaCl₂, 0.05 M-Tris, 0.1 M-NaCl, pH 7.5). Trypsin activity was blocked with soya bean trypsin inhibitor and stromelysin activity was stopped by freezing samples at −20°C until digested fragments were separated from the proteinase by gel filtra-

Abbreviations used: PG, proteoglycan; PMSF, phenylmethyl-sulphonylfluoride.

Fig. 1. Uptake of PG and PG fragments by THP-1 cells
(a) Gel filtration profiles on sepharose CL-6B of whole PG aggregate (●); PG after digestion with stromelysin (▲); and PG after digestion with trypsin (■). (b-d) Uptake of selected CL-6B fractions by THP-1 cells. Hatched bars show material removed from the cells by acetate buffer, pH 3.5; open bars show material remaining cell associated. (b) Undigested PG aggregate; (c) stromelysin-digested PG; (d) trypsin-digested PG.

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tion on Sepharose CL-6B (see Fig. 1e). Aliquots of iodinated PG and selected proteolytic fragments were incubated with the human monocytic cell line THP-1 [19] using 5 × 10³ c.p.m. and 3 × 10⁴ cells. The location of PG in the cells after uptake was determined after disruption by N₂ cavitation followed by subcellular fractionation on Percoll gradients (1.05 g/ml starting density) [20]. Hexosaminidase and 5'-nucleotidase activities were used as markers for lysosomes and plasma membrane, respectively.

The amount of PG taken up by the THP-1 cells continued to increase with time for the first 45 min after which it reached a plateau representing 5% of the initial total label. Pretreatment of the cells with the serine proteinase inhibitor phenylmethylsulphonylfluoride (PMSF) or lowering the temperature to 4°C reduced uptake by 20% and 40%, respectively. Ninety percent of the PG taken up during 60 min of incubation was resistant to stripping in 0.1 M-sodium acetate, 0.15 M-NaCl buffer, pH 3.5, and was presumably therefore intracellular. After a further 60 min chase in fresh medium, 50% of this had been secreted back into the medium. Gel chromatography showed that this secreted PG had been extensively degraded, so that only 20% was excluded from Sepharose CL-6B compared with 100% of the original material. The remaining 80% contained a polydisperse range of fragments ranging in size from a few kDa to several hundred kDa. Subcellular fractionation of disrupted cells on Percoll after uptake for 60 min confirmed that 81% of the PG was contained within lysosomes, while only 19% was membrane associated. Samples of PG that had been digested with trypsin or stromelysin were chromatographed on Sepharose CL-6B (Fig. 1) and selected fractions from each profile tested for uptake by THP-1 cells. Cells were then stripped in acetate buffer, pH 3.5, to determine the percentage of this label that remained surface associated. Some fractions were taken up much more rapidly than others (Fig. 1) and a high proportion of these fragments were removed from the cells by the acid buffer in contrast to the results obtained with whole PG aggregate. In particular the rate of uptake of fraction 70 (Kₜ = 0.77; Fig. 1) was very rapid, a plateau of surface-bound material being achieved within 2–4 min incubation. When THP-1 cells were pulsed for 5 min with fraction 70 and then chased in fresh medium, the amount of cell-associated label declined to about 15% of the initial value within 30 min. The remaining surface-associated label, however, was stable over a period of 2 h and remained susceptible to stripping at low pH. Uptake of these fragments was reduced greatly to only 20% of normal levels at 4°C or in the presence of PMSF. When whole PG aggregate and proteolytic PG fragments were offered to resident mouse peritoneal macrophages, which do not usually express class II antigens [21], whole aggregate was taken up at a rate similar to that seen with THP-1 cells and was also resistant to stripping in acid buffer. In contrast, mouse macrophages took up less than 0.2% of the PG fragments over a period of 5 min.

These very different rates of uptake, the different sensitivities to temperature and PMSF, and the different affinities shown by macrophages and THP-1 cells, suggest that the mechanism of recognition and uptake of the proteolytic fragments is different from that of whole PG aggregate. Experiments are in progress to determine whether these acid-strippable PG fragments are associated with class II antigens on the THP-1 cell membrane.

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Effect of interleukin-1 and tumour necrosis factor-α on the turnover of proteoglycans in human articular cartilage

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The entrapment of proteoglycans within a collagen network endows cartilage with its compressive properties and prevents transmission of stresses to the subchondral bone. Regulation of proteoglycan metabolism is maintained by the continuous synthesis and turnover of these components by the chondrocytes. How these processes are controlled is unknown, but they probably involve the response of the cells to changes in their matrix and to local and systemic hormones and cytokines. Cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) stimulate proteinase production and induce the resorption of cartilage; they also inhibit the synthesis of aggregating proteoglycan by the chondrocytes [1, 2]. These results have mainly been

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