GRANZYME B EXPRESSION IN ARTICULAR CARILAGE

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Introduction
Granzyme B is a serine proteinase that is involved in granule mediated apoptosis1,2. Recent findings suggest that human granzyme B degrades proteoglycan aggrecan3. Granzyme B, produced by natural killer cells (NK) and cytotoxic T cells (CTL), is detected in synovial tissues and fluids from patients with rheumatoid arthritis(RA)4,5, whereas existence of the granzyme B in chondrocytes still remain unclear. The purpose of this study was to investigate the expression of granzyme B in the articular cartilage of RA, osteoarthritis (OA) and normal on both the messenger RNA (mRNA) level and the protein level.

Materials and Methods

Human RA and OA cartilage was obtained from condyle at knee joint replacement surgery from 18 RA patients and 10 OA patients. All RA patients satisfied the 1987 American College of Rheumatology criteria for RA. Normal human cartilage, which defined as control, was obtained from femoral head at joint replacement surgery from 10 patients who had no history of joint disease. A part of cartilage tissue were used for RNA isolation. The rest of the tissue was used for immunohistochemistry. Positive control for reverse transcriptase-polymerase chain reaction (RT-PCR) was lymphokine-activated killer cells (LAK). LAK were prepared as following. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood of healthy individual by Ficoll density–gradient centrifugation. Isolated PBMC were cultured with interleukin 2 (Genzyme, MA, USA) for 5 days.

Molecular analysis: The cartilage tissue, which were removed from condyle or femoral head, were incubated on a gyratory shaker at 37 °C with a digestion buffer containing collagenase(type II) until the fragment were digested. Experiments of chondrocyte reported here were performed with primary cells. Total RNA was extracted using the Trizol Reagent (GibcoBRL, Connecticut, USA). Total RNA was into complementary DNA (cDNA) using SuperScript reverse transcriptase (GibcoBRL, Gaitherburg, MD) and oligo(dT)15 as primer. The detection of granzyme B mRNA was performed using PCR experiments with Taq DNA polymerase (Boehringer Mannheim,USA). The concentration of cDNA was adusted by Human -actin competitive PCR set (Takara, Kyoto, Japan). Granzyme B mRNA was qualitatively determined by competitive PCR experiments. They were performed using the competitor which were produced by using Competitive DNA Construction Kit (Takara, Kyoto, Japan). We analyzed the products by electrophoresis in 1.5% agarose gels and photographed. The resulting photographs were scanned using page scanner, and the images were analyzed using NIH image software to determine the pixel intensity value for each band. The values of granzyme B mRNA in RA, OA and normal cartilage were compared.

Immunohistochemistry: The cartilage tissue samples were immediately fixed in 4% paraformaldehyde, and processed for paraffin embedding. 5 μm sections were immunostained with a human granzyme B monoclonal antibody (Pharma Cell, Paris, France). Sections were incubated with the primary antibody at a 1:1/3 dilution overnight at 4 °C. And the numbers of cells that expressed granzyme B were compared. Kruskal Wallis test was used in the statistical analysis, and a probability value of less than 5% was considered significant.

Results

Figure 1 shows that expression of granzyme B mRNA was detected in chondrocytes from all cases of RA, OA and normal except one sample. The mRNA levels for granzyme B showed that the value in RA was approximately 8-fold higher than in OA, and 32-fold higher than in normal with significant difference. Figure 2 shows expression of granzyme B protein in chondrocytes. In RA, granzyme B expressing chondrocytes were mainly in the superficial and deep layer. Furthermore, in deep layer, they were in the vicinity of pannus that have invaded from the bone marrow. In OA and normal cartilage, almost all of granzyme B expressing chondrocytes were in the superficial layer. Figure 3 shows that there were significant differences between the numbers of granzyme B expressing chondrocytes in RA, OA, and normal cartilage.

Discussion
Granzyme B chiefly exists in the intracelular granules of NK and CTL. We demonstrated that the presence of granzyme B in chondrocytes. Furthermore the level of granzyme B was markedly elevated in RA chondrocytes. Granzyme B has two functions which are apoptosis induction and degradation of proteoglycan aggrecan. In RA, granzyme B production in a chondrocyte may be involved in apoptosis itself, if it is in the chondrocyte. And granzyme B may be involved in extracellular matrix destruction, if it is released extracellular space. The data presented here may also provide evidence implicating granzyme B in the pathogenesis, probably cartilage autolysis, of RA. On the other hand, since granzyme B was present in normal chondocytes, it is possible that it plays some role in maintaining the homeostasis of cartilage tissue under normal conditions.

Reference