SIGNAL TRANSDUCTION PATHWAY AND APOPTOSIS IN BACTERIA-INFECTED CHONDROCYTES

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Introduction In joints with bacterial arthritis, continuing prolonged destruction of cartilage may occur in spite of prompt and effective antibiotic therapy. Synovial and systemic upregulation of cytokine mRNA expression during Staphylococcus aureus arthritis has been demonstrated by in situ hybridization. Bacterial toxins and cytokines are known inducers of apoptosis, which might be responsible for the hypocellularity commonly seen in septic arthritis. However, the mechanism of apoptosis in bacteria-infected chondrocytes remains unclear. The purpose of this study was to investigate the induction of apoptosis in bacteria-infected chondrocytes and to examine the signal transduction pathways activated by bacteria infection.

Materials and Methods Primary cultures of human articular chondrocytes were challenged with Staph. aureus (ATCC29213) in a dosage of one bacteria per one cell. DNA fragmentation was analyzed by flow cytometry. Infected chondrocytes were harvested at different time intervals and resuspended in 1.5 ml hypotonic fluorochrome solution. The red fluorescence of nuclei was recorded and expressed as the percentage of apoptotic (hypodiploid) nuclei. Detection of phosphatidylserine externalization was carried out using fluorescence microscopy. Chondrocytes were cultured in Lab-Tek chamber slides and challenged with Staph. aureus as above. Cells were labelled at defined intervals with Annexin V-FITC. Infected chondrocytes were also immobilized onto glass slides by using a Cytospin®. The internucleosomal cleaved nucleotide ends were labeled in apoptotic cells using biotin-conjugated deoxynucleotides by terminal deoxynucleotidyl transferase (TUNEL assay). Detection of signal transduction pathway was carried out using co-transfection technique. Chondrocytes were co-transfected with fusion activator plasmids and reporter plasmid using LipofectAMINE reagent (Gibco). The signal pathway was detected by PathDetect™ in vivo signal transduction pathway reporting systems (Stratagene). After a 24-hour of transfection, cells were challenged with bacteria for 6 hours and then were subjected to extraction of intracellular luciferase activity. Nitric oxide (nitrite) production was detected in the culture media samples by Griess reaction with sodium nitrite as standard.

Results Phosphatidylserine externalization was observed in cells after bacterial infection (fig 1). Bacterial infection resulted in a progressive increase in the number of hypodiploid cells as detected by flow cytometry (fig 2). Normally, there was 5.4±1.2% of cells with fragmented DNA. After infection for 2 hours, it increased to 9.5±3.0%. At 6 hours, there was 31.0±6.2%. At 10 hours, apoptotic cells accounted for 48.0±6.9% of the totally counted cells (fig 3). Semiquantitative analysis of internucleosomal cleavage of chromosomal DNA indicated that 8.1% of cells were TUNEL positive in untreated group; in contrast to 54.7% of bacteria-infected chondrocytes after a 24-hour incubation. All three signal pathways activity were increased after bacterial infection. The JNK activity increased 7.6 times the negative control, the MAPK activity increased 7.3 times the negative control, and the PKA activity increased 3.2 times the negative control. The nitrite level after bacteria infection was increased with increasing incubation time (fig 3).

Discussion In this study, we provided evidence that upto 55% of chondrocytes undergo apoptosis with a 24 hour infection by a single organism. The chondrocytes firstly externalized phosphatidylserine and their nucleosomal DNA were cleaved by endonuclease upon co-incubation with bacteria within 2 hours. The cellular membrane remained intact up to 6 hours of co-incubation and then gradually lost integrity with increasing incubation time.

Nitrergic is a potent inducer of apoptosis in cultured human articular chondrocytes. Articular chondrocytes can synthesize nitric oxide in response to IL-1, TNF-α, and lipopolysaccharide. Induction of high level of chondrocytic nitric oxide during infection or inflammation may be responsible for the damage to cartilage that occurs in some inflammatory arthropathies. In this study, we also found that the nitrite level increased 10 fold after a 10-hour incubation relative to the baseline nitrite production in uninfected cells. However, by using the competitive inhibitor of nitric oxide, N-monomethyl-L-arginine, there was no significant suppression of the amount of apoptotic cells (data not shown). These findings suggested the causes of apoptosis in bacteria-infected chondrocytes are multifactorial. The signal pathways involved increased activity of JNK, MAPK, and PKA. The JNK activity was higher in the infection samples than the postive control samples but either MAPK or PKA activity after infection had higher activity than positive control.

In conclusion, the human chondrocytes undergo apoptosis at an early stage of bacteria infection. The injured cells gradually lost their membrane integrity if the co-incubation time exceeded 6 hours. These findings can partly explain why early administration of antibiotic and lavage of joint are not able to prevent chronic and sustained destruction in septic arthritis. Accordingly, if we can inhibit apoptosis in bacteria-infected chondrocytes at early stage, the long term sequela can possibly be prevented.

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