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Gene therapy with NF-κB decoy oligodeoxynucleotides in arthritis

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Keywords

Collagen-induced arthritis, gene therapy, NF-κB, transcription factors

Context

One of the best studied transcription factors, NF-κB, is involved in regulating the expression of many inflammatory proteins. Upon cell stimulation, NF-κB dissociates from the cytoplasmic inhibitory molecule IκB, then translocates into the nucleus where it binds specific DNA sequences in the promoters of target genes, and activates their transcription. Genes regulated by NF-κB include those encoding some adhesion markers, chemokines and pro-inflammatory cytokines (such as interleukin [IL]-1β, IL-6 and tumour necrosis factor [TNF]-α). Consequently, the activity of NF-κB is a logical target for therapeutic strategies against rheumatoid arthritis (RA), and NFκB's natural inhibitor, IκB, has been utilised in a number of studies. Here, Tomita *et al*target NF-κB activity by interfering with its binding to promoter sequences. Introduction of chemically modified, nuclease resistant decoy oligodeoxynucleotides (ODN) containing the NF-κB recognition sequence results in the transcription factor binding this exogenous DNA, leaving the regulatory elements upstream of the target genes nonactivated. To inhibit the activity of NF-κB locally in the arthritic joint using DNA decoys and, consequently, to limit expression of inflammatory genes and the progression of collagen-induced arthritis (CIA).

Significant findings

*In vivo*transfection of FITC-labelled ODN by intra-articular injection into inflamed joints caused a wide distribution of fluorescence in the joint synovium. This was primarily localised to the nuclei of synovial cells, and inclusion of the HVJ-liposome was required for a strong and sustained signal. Following immunohistochemistry and fluorescence microscopy, the transfected cells were identified as macrophage- and fibroblast-like and remained fluorescent for at least 25 days. NF-κB was locally activated in the arthritic joint, as determined by gel mobility shift assay, where an almost four fold increase in NF-κB binding in the diseased joint was observed compared to healthy tissue. The local inflammation, measured as paw swelling, was efficiently inhibited following NF-κB ODN treatment, whereas a scrambled ODN did not have any ameliorative effect. The beneficial result was localised, hence the nontreated front paws followed the arthritic progression observed in control animals.

Radiographic examinations showed no definite structural destruction in the treated joints and the cellular infiltration of synovium was reduced, as assessed by histology. The concentration of IL-1β and TNF-α in the joint was significantly reduced, correlating with their mRNA levels.

Comments

Instead of tackling the biological effects of pro-inflammatory factors, this paper reports on the therapeutic outcome of inhibiting their local expression by blocking the activity of nuclear factor (NF)-κB by decoy oligodeoxynucleotides (ODN). The results are impressive, with a reduction in paw swelling, joint destruction and expression of pro-inflammatory cytokines. The finding of this study extended those of Makarov et al (Proc Natl Acad Sci 1999, **95**:13859-13864 [Abstract]), a reference overlooked by Tomita et al. The novelty of this study is the introduction of a viral protein in the decoy complex, which increases the transfection efficacy.

A basal level of active NF-κB was detected in the normal joints of the arthritis model used. This raises the question of whether inhibiting the ubiquitous effects of NF-κB would specifically prevent inflammatory events without affecting possible housekeeping roles of the transcription factor. It would be interesting to see how the expression of other genes were affected following the ODN treatment.

Methods

The haemagglutinating virus of Japan (HVJ)-liposome method was used to effectively transfer phosphothioate modified ODN into the target cells, where the viral component assists membrane fusion, thus allowing entry of the foreign complex. The efficacy of transfection *in vivo* was assessed by intra-articular injection of fluorescein isothiocyanate (FITC)-labelled ODN complexes into rats, and compared with that of ODN delivered without the vector. The animals were sacrificed at different time points and the fluorescence of synovium was measured. To localise FITC-ODN intracellularly, ultraviolet confocal microscopy was performed on cryostat sections. These were also stained immunohistochemically for the detection of macrophage markers. CIA was induced by immunising the animals with bovine collagen type II. To assess the NF-κB activity in the synovium of arthritic rats, a gel mobility shift assay was carried out on nuclear extracts of synovial cells using ^{32}P labelled NF-κB ODN as probe. Two weeks after immunisation, scrambled or NF-κB specific ODN, complexed with HVJ-liposome, was injected into the inflamed hind ankle of rats. The course of CIA was monitored by measuring the hindpaw swelling. At the end of the experiment, radiological and histological studies were carried out. The concentration of IL-1β and TNF-α was measured in synovial fluid 1 and 7 weeks after transfer. Amounts of IL-1β and TNF-α RNA transcripts in synovial tissue were estimated by northern blotting.

References

1. Tomita T, Takeuchi E, Tomita N, Morishita R, Kaneko M, Yamamoto K, Nakase T, Seki H, Kato K, Kaneda Y, Ochi T: Suppressed severity of collagen-induced arthritis by *in vivo*transfection of NFκB decoy oligodeoxynucleotides as a gene therapy. Arthritis Rheum. 2000, 42: 2532-2542.