8. Towards novel therapeutic strategies

A158 TOWARDS GENE THERAPY FOR THE TREATMENT OF RHEUMATOID ARTHRITIS: PRODUCTION AND BIOACTIVITY OF INTERFERON β IN FIBROBLAST-LIKE SYNOVIOCYTES TRANSDUCED WITH ADENO-ASSOCIATED VIRUS TYPE 5 EXPRESSING HUMAN INTERFERON β

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Background and objectives The authors are developing local intra-articular gene therapy for the treatment of rheumatoid arthritis (RA) using human interferon β (hIFNβ) as therapeutic gene expressed under control of an inflammation-inducible promoter and recombinant adeno-associated virus type 5 as vector (ART-I02). To evaluate hIFNβ expression by ART-I02 and bioactivity, fibroblast-like synoviocytes (FLS) from RA patients and different animal species were transduced with ART-I02 and the culture medium was analysed. Since most RA patients in their future phase I trial will be on methotrexate (MTX) treatment, the authors investigated the influence of MTX on transduction efficacy.

Methods Primary human, monkey, mouse and rat and non-primary rabbit FLS were transduced with ART-I02 (MOI 200.000). To activate the nuclear factor-κB promoter, cells were stimulated with tumour necrosis factor (TNF) (1 ng/ml) with or without interleukin 1β (IL-1β) (10 ng/ml) 4 or 24 h post-transduction. In addition, human FLS were incubated in medium with MTX (10 nM, 1 μM, 100 μM) added pretransduction and post-transduction. Supernatants were harvested 48 h after stimulation. Production of hIFNβ was measured by ELISA. Bioactivity was established by analysing the effect on pro-inflammatory cytokine (IL-6, IL-8) and matrix metalloproteinases (MMP) production by ELISA and with a quantitative gene reporter bioassay.

Results Human IFNβ production was detectable in supernatants of FLS of all species, with comparable levels in human and monkey FLS. In human FLS transduced with ART-I02, the inhibition of IL-8 (80%) and MMP3 (60%) secretion was most pronounced after stimulation with TNF 24 h after transduction ($p<0.05$). IL-6 production was significantly ($p<0.05$) reduced by hIFNβ in cells stimulated with both TNF and IL-1β. Monkey FLS expressing hIFNβ showed a 40% decrease in IL-8 secretion ($p<0.01$) after stimulation with TNF. No effect of hIFNβ on cytokine and MMP secretion was observed in rabbit and rodent FLS. Human IFNβ produced by both human and monkey FLS showed robust levels of bioactivity in the gene reporter bioassay. MTX treatment did not influence hIFNβ production by ART-I02 or bioactivity.

Conclusion Transduction of FLS with ART-I02 resulted in significant hIFNβ expression in FLS of all species. Moreover, hIFNβ produced by ART-I02-transduced cells is bioactive in human and monkey FLS. This study supports the use of non-human primates in a non-clinical pharmacology-toxicity programme and represents a next step towards a phase I clinical trial in RA patients.