Summary & Conclusions
Rheumatoid arthritis (RA) is an autoimmune disease, characterized by chronic inflammation in the joints and subsequent destruction of the cartilage and bone. The etiology of RA is still unknown, but it has been recognized that many cytokines are up-regulated in RA synovium which correlates with the joint lesions. In particular, tumor necrosis factor α (TNF-α) has been identified in the pathogenesis of RA, not only for its ability to directly induce cartilage and bone degradation but also for its capacity to initiate an inflammatory response. Thus, TNF-α promotes secretion of other inflammatory mediators, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and granulocyte macrophage colony stimulating factor. Accordingly, blockade of TNF-α with monoclonal antibodies, downregulates the production of these cytokines in RA synovial tissue cultures. Anti-TNF-α antibodies have also been demonstrated to suppress joint inflammation in collagen-induced arthritis, a murine model of RA. Furthermore, mice expressing human TNF-α transgenes develop chronic, progressive polyarthritis that is prevented by treatment with anti-TNF-α antibodies. Altogether these findings have contributed to the emerging concept that TNF-α is a good therapeutic target in RA. Treatment with monoclonal antibodies or soluble receptors to TNF-α show significant clinical benefit in RA. Although having clinical efficacy, these agents have significant disadvantages, such as the need for repeated high doses and the potential of inducing allergic reactions or immune responses to the protein-based drug. An alternative method would be to inhibit TNF-α production at the gene transcriptional level. Inhibition of TNF-α mRNA production may be a more efficient method in treating conditions associated with its overexpression.

One approach to block gene expression is through the use of ribozymes. Ribozymes are catalytic RNA molecules possessing the ability for specific cleavage of target RNA. They function by binding with target RNA moiety through Watson Crick base pairing and inactivate it by cleaving the phosphodiester backbone. Development of this ribozyme strategy has been successful in inhibiting gene expression in cell cultures. However, data are still limited for the efficacy of ribozymes in vivo, especially in disease models. Here we have for the first time investigated the therapeutic activity of a ribozyme in a model of autoimmune arthritis. We designed a hammerhead ribozyme against murine TNF-α that could specifically cleave TNF-α mRNA and inhibit LPS-induced TNF-α production in cell culture. The therapeutic potential of the ribozyme in
vivo was tested by systemic delivery of plasmid DNA to mice with collagen-induced arthritis. We report that ribozyme targeting TNF-α mRNA has a substantial therapeutic effect on arthritis, thus indicating its potential clinical use to control TNF-α dependent chronic inflammatory conditions of autoimmune origin. In this study we show that RNA molecules (ribozymes) possess therapeutic activity on inflammatory processes in vivo, as judged from effects on an arthritis model in mice.

**Design and Cleavage of Murine TNF-α mRNA with Cognate Ribozyme**

Ribozyme was designed against mTNF-α by using computer programs (FOLDRNA, MFOLD and SQUIGGLES, UNIX Version 9.1, GCG, Wisconsin Package) based on Zuker’s algorithm of energy minimization. Designed ribozyme could efficiently and specifically cleave murine TNF-α in vitro.

**Ribozyme suppresses TNF-α mRNA in LPS stimulated Cells**

Ribozyme designed against tumor necrosis factor alpha was verified for its catalytic activity by transfection into a macrophage cell line. The ribozyme expression construct inhibited production of lipopolysaccaride-induced tumor necrosis factor alpha mRNA in the cells and lowered the titer of apoptotic factor in culture supernatant.

**In Vivo Ribozyme Expression and Amelioration of CIA**

When administered systemically in vivo, the ribozyme significantly reduced the development of collagen-induced arthritis in mice. No effect was observed with a catalytically inactive variant of the ribozyme. Furthermore, the ribozyme blocked cartilage and bone destruction in the joints and efficiently ameliorated established CIA. This study shows for the first time suppression of autoimmune arthritis by a ribozyme directed tumor necrosis factor alpha mRNA in vivo, thus providing proof of concept that it may be used as therapeutic tool for rheumatoid arthritis.

To sum up, these studies demonstrate the power of using ribozyme based gene targeting of TNF-α in vivo for modulating autoimmune arthritis and point out the potentials of the ribozyme against TNF-α as therapeutic tool for the management of RA and other TNF-α related diseases.
Conclusions

- Ribozyme was designed against murine TNF-α mRNA based on secondary structure considerations.

- Designed ribozyme was cloned into pStu I and pCI-neo vectors and confirmed by restriction digestion and sequencing.

- Murine TNF-α cDNA was subcloned in pStuI vector.

- Ribozyme-mediated cleavage of TNF-α mRNA, \textit{in vitro}, indicates that the designed ribozyme has efficiently and specifically cleaved the target.

- The ribozyme apparently assumes novel conformations in the presence of glucose-6-phosphatase with different electrophoretic mobility.

- TNF-α targeted ribozyme needs magnesium ions for its catalytic activity as many other catalytic RNAs do.

- Expression of ribozyme in J774A.1 cells stimulated with LPS shows reduced level of TNF-α mRNA as evident from ribonuclease protection assay.

- Expression of ribozyme in J774A.1 cell stimulated with LPS shows reduced amount of TNF-α at protein level as the culture supernatant was assayed on WEHI-164 cells for apoptosis.

- When administered systemically in vivo, the ribozyme significantly reduced the development of collagen-induced arthritis in mice.