Disease-regulated Local Interleukin-10 Gene Therapy Diminishes Synovitis and Articular Cartilage Damage in Experimental Arthritis

Eline Vermeij, Mathijs Broeren, Miranda Bennink, Onno Arntz, Inger Gjertsson, Wim van den Berg, Fons van de Loo

Department of Experimental Rheumatology

Radboud university medical center, Nijmegen, The Netherlands
Rheumatoid Arthritis (RA)

- Disease course with flares and remission

- Conventional treatment includes biological drugs:
  - repeated administration
  - systemic

- Goal: Gene therapy using disease-inducible promoters
  - local
  - only active during flare
Disease-inducible promoters

- Microarray on joints of mice with experimental arthritis
- Find genes upregulated in arthritic mouse joints
- Isolate promoter from endogenous gene

**Question**: Which promoter is suitable for gene therapy?

Geurts et al. 2009
In-vivo profiling of inducible promoters

- 300 ng lentivirus intra-articular in knee joint
- Induction SCW arthritis 4 days after transduction
- Imaging at day 0, 1, 4, 7 and 9
Kinetics of inducible promoter reporters

- **S100A8**
- **CXCL1**
- **MMP13**
- **SAA3**
- **IL-1b**
- **TNFaip6**
Kinetics of inducible promoter reporters

- Saa3 promoter was selected
  - Highest fold induction
  - Early peak at day 1 after arthritis induction
- Transgene → replace luciferase by Interleukin-10
Transgene → Interleukin-10 (IL-10)

- IL-10 is a broad spectrum anti-inflammatory cytokine
  - Produced by many inflammatory cells
  - Inhibits production of several pro-inflammatory cytokines
  - Induces production of anti-inflammatory cytokines
  - Short half-life in serum: between 1.1 – 2.6 hours

![Graph showing arthritic index over days after the first immunization](image)

**PBS treatment**
- IL-10 day 21-48
- IL-10 day 0-48
- IL-10 day 0-21

Tanaka et al. 1996
Saa3 promoter response to IL-10

- Stimulation of lentiviral transduced NIH-3T3 fibroblast cells
  - Transduced with Saa3-Luc (50 ng p24\textsuperscript{gag} equivalents/well)
  - Stimulated for 6 hours with IL-10 (10 ng/ml), SCW (5 µg/ml) or combination
- IL-10 stimulation did not upregulate the Saa3 promoter
  - Inhibition of SCW stimulation with IL-10
Experimental setup in-vivo experiment

• Day -4 = i.a. injection lentivirus (300 ng)
  • PGK-Empty (virus control)
  • PGK-IL10 (positive control)
  • Saa3-IL10
• Day 0 = i.a. injection SCW (25 µg)
• Day 1, 4, 7 = isolation knee joint / synovium for histology or RNA isolation + serum for cytokine analysis
IL-10 overexpression with inducible Saa3 promoter

- Transgene expression at day 1, 4 and 7 in the arthritic joint
- IL-10 expression at all days → Saa3 promoter is upregulated

![Graphs showing dCT values for Empty, PGK, and Saa3 groups at days 1, 4, and 7.](image-url)
**IL-10 overexpression with inducible Saa3 promoter**

- Transgene expression at day 1, 4 and 7 in the arthritic joint
  - IL-10 expression at all days → Saa3 promoter is upregulated

![Graph showing transgene expression](image)

- Transgene expression at day 1 in the arthritic and contralateral non-arthritic joint
  - Saa3 promoter shows inducible production of IL-10

![Graph showing spontaneous and inducible production](image)
IL-10 expression reduces synovitis

Day 4 after SCW

- Synovitis decreased at day 4
Cartilage damage is reduced at day 4 and 7

- Proteoglycan depletion decreased at day 4 and 7
Effects of IL-10 overexpression on synovial chemokine production

- KC protein downregulated at day 1 after SCW injection
  - Important chemokine in the pathogenesis of arthritis
Both IL1RA and SOCS3 are upregulated
- **IL1RA upregulation**
  - Counteracts detrimental effects of IL-1 on cartilage damage → less proteoglycan depletion\(^1\)

- **SOCS 3 upregulation**
  - inhibits JAK/STAT pathway and subsequent inflammation → less synovitis\(^2\)

1) Kuiper et al., 1998
2) Henningsson et al., 2012
Implications for gene therapy in RA

• The Saa3 promoter is inducible in experimental arthritis

• Saa3p-IL10 gene therapy can diminish synovitis and proteoglycan depletion

• Saa3p-IL10 gene therapy can upregulate important anti-inflammatory cytokines and genes

• **Saa3 promoter is a good candidate for gene therapy using IL-10**
Acknowledgements

Department of Rheumatology
Radboud University Medical Centre
Nijmegen, Netherlands

- E. A. Vermeij
- M.B. Bennink
- A.J. Arntz
- F. A.J. van de Loo
- I. Gjertsson
- W.B. Van den Berg