Intra-articular overexpression of interleukin-10 using disease-inducible promoters diminished synovitis and cartilage proteoglycan depletion in experimental arthritis

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Rheumatoid Arthritis

• In about 30% of the RA patients, the disease course is characterized by an intermittent pattern of exacerbation and complete remission

• Conventional treatments
  • Repeated administration
  • Side effects

• Gene therapy with disease-inducible promoters
Disease-inducible promoters

- Selection of promoters from endogenous genes differentially regulated in synovium of collagen induced arthritis mice by computational approach

- Disease-inducible promoters:
  - Only produce a therapeutic gene during flare of disease
    - No production during remission, so less side effects
  - Local treatment

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 6 different inducible promoter reporters

- **S100A8**
- **CXCL1**
- **MMP13**
- **SAA3**
- **IL-1b**
- **TNFaip6**
Kinetics of 6 different inducible promoter reporters

- Saa3 promoter was selected
  - Highest fold induction
  - Early peak at day 1 after arthritis induction
- Transgene \(\rightarrow\) Interleukin-10
Transgene → Interleukin-10

- IL-10 is a broad spectrum anti-inflammatory cytokine
  - Produced by Th1 and -2 cells, B-cells, monocytes, macrophages
  - Inhibits production of several pro-inflammatory cytokines
  - Induces production of IL-1 receptor antagonist (IL1RA)
  - Short half-life in serum: between 1.1 – 2.6 hours

Tanaka et al., 1996
Saa3 promoter did not react to IL-10

- Stimulation of lentiviral transduced NIH-3T3 fibroblast cells
  - Transduced with Saa3-Luc (50 ng p24\textsubscript{gag} equivalents/well)
  - Stimulated for 6 hours with IL-10 (10 ng/ml), SCW (5µg/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 (25µg)
- Day 1,4,7 = isolation knee joint / synovium for histology or RNA isolation + serum for cytokine analysis
IL-10 overexpression

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

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

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

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

- Transgene expression at day 1 in the arthritic and contralateral non-arthritic joint
  - Saa3 promoter shows inducible production of IL-10
Less synovitis can be seen at day 4

Day 4 after SCW
Cartilage damage is diminished at day 4 and 7

Day 7 after SCW

Day 4

Day 7
Effects of IL-10 overexpression on synovial cytokine production and gene expression

- IL-8 protein was significantly downregulated at day 1 after SCW injection
- Chemokine that plays an important role in the pathogenesis of arthritis
Both IL1RA and SOCS3 are upregulated
- SOCS 3 upregulation
  - inhibits JAK/STAT pathway and subsequent inflammation → less synovitis (Henningsson et al., 2012)
- IL1RA upregulation
  - Counteracts detrimental effects of IL-1 on cartilage damage → less PG depletion (Kuiper et al., 1998)

Effects of IL-10 overexpression on synovial cytokine production and gene expression
MMP-13 promoter

- MMP13 promoter is also upregulated, but not according to profile
  - Influence of IL-10 on MMP13 expression profile
Implications for gene therapy in RA

• Inducible promoters Saa3 and MMP13 both showed effects on synovitis and PG depletion
  • Choice of inducible promoter also depends on the transgene
    • Important to know whether promoter is regulated by transgene

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