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

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No disclosures
Rheumatoid Arthritis

- The majority of RA patients (70%) show progression of disease, often with pauses.

- About 15% of people with rheumatoid arthritis have disease that waxes and wanes slowly.

- Conventional treatment includes biological drugs:
  - repeated administration – invasive
  - systemic administration - side effects
  - long-term treatment - even during remission
Objective

To develop a gene therapeutic approach for disease-regulated delivery of biologics

- local delivery - viral transduction synovium/resident cells
- Long-term expression – mammalian promoters/ integrating vectors
- Only production during active disease - promoters of inflammation reactive genes
Search promoters of disease-inducible genes

- Microarray of synovial tissue of mice with collagen induced arthritis
- Selection of genes upregulated during arthritis
- Prediction of regulatory elements on their transcriptional promoter
- Clone proximal promoter into viral expression vectors
Validation: in-vivo profiling of selected 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 promoter-luciferase expression
Promoter of serum amyloid A3 was selected

- Highest fold induction (120x)
- Rapid activation at day 1 of arthritis
- Reporter expression remains high during synovitis

Next: Replace luciferase transgene for an antiinflammatory gene
Saa3-regulated IL-10 gene therapy

IL-10 has pleiotropic anti-inflammatory effects:

- Produced by Th1, Th2, B-cells, monocytes, macrophages
- Inhibits antigen-presentation (MHCII, costimulatory antigens)
- Capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF
- Can block NF-kB and STAT-activation
- SOCS3 and IL-1Ra
- Short half-life in serum: between 1.1 – 2.6 hours
To prevent uncontrolled production the Saa3 promoter should not be activated by IL-10

- Stimulation of lentiviral transduced NIH-3T3 fibroblast cells
  - Transduced with LV.Saa3-Luc (50 ng p24gag equivalents/well)
  - Stimulated for 6 hours with IL-10 (10 ng/ml), SCW (5µg/ml) or combination
  - IL-10 did not activate the Saa3 promoter

![Graph showing RLU levels for Medium, IL-10, SCW, and IL-10/SCW](image)
Experimental setup arthritis experiment

- Day -4 = i.a. injection lentivirus (300 ng p24)
  - PGK-Empty (virus control, Phosphoglycerate kinase promoter)
  - 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 RNA 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

- Saa3 promoter shows selective and inducible response in the arthritic joint
Histology at day 4

Day 4 after SCW

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

- Proteoglycan (PG) loss decreased at day 4 and 7
Effects of IL-10 overexpression on synovial cytokine production and gene expression

- Reduced IL-8 (KC) production at day 1 of arthritis by IL-10 overexpression
  - A neutrophil attractant that plays an important role in pathogenesis of arthritis
IL-10 induced synovial expression of IL-1Ra and SOCS3

- SocS3 inhibits JAK/STAT pathway and subsequent inflammation → less synovitis (Henningsson et al., 2012)

- IL1Ra counteracts detrimental effects of IL-1 on cartilage damage → less PG depletion (Kuiper et al., 1998)
Endogenous IL-10 is expressed early in disease!

**TABLE 1. Joint swelling, inhibition of cartilage PG synthesis and levels of cytokines during SCW arthritis**

<table>
<thead>
<tr>
<th></th>
<th>Joint swelling (R/L ratio)</th>
<th>Inhibition of PG synthesis</th>
<th>IL-1β (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>mIL-10 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 h</td>
<td>ND</td>
<td>ND</td>
<td>100 ± 20</td>
<td>420 ± 50</td>
<td>&lt;4</td>
</tr>
<tr>
<td>6 h</td>
<td>ND</td>
<td>−2 ± 4%</td>
<td>1190 ± 390</td>
<td>180 ± 40</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>day 1</td>
<td>1.81 ± 0.11</td>
<td>−49 ± 6%</td>
<td>490 ± 120</td>
<td>&lt;40</td>
<td>&lt;4</td>
</tr>
<tr>
<td>day 2</td>
<td>1.49 ± 0.04</td>
<td>−43 ± 5%</td>
<td>150 ± 20</td>
<td>&lt;40</td>
<td>&lt;4</td>
</tr>
<tr>
<td>day 4</td>
<td>1.21 ± 0.09</td>
<td>−21 ± 4%</td>
<td>120 ± 17</td>
<td>&lt;40</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

Unilateral arthritis was induced by intraarticular injection of 25 μg SCW into the right knee joint of naive mice. Joint inflammation was quantified by the $^{99m}$Tc uptake method and the chondrocyte PG synthesis was assessed in patellae by $^{35}$SO$_2^-$ incorporation ex vivo as described in Materials and Methods. The levels of IL-1β, TNF-α, and IL-10 in patellae washouts were measured by radio-immunoassays (RIA) and ELISA, with a detection limit of 20, 40 and 4 pg/ml, respectively. (ND = not done).

MMP13-IL10 could be as effective
MMP13-IL10

SCW control virus

SCW MMP13-IL10 virus

e
Histological score

<table>
<thead>
<tr>
<th>Day 4 syn</th>
<th>Day 7 syn</th>
<th>Day 4 PG dep</th>
<th>Day 7 PG dep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2.0</strong></td>
<td><strong>1.5</strong></td>
<td><strong>1.8</strong></td>
<td><strong>1.4</strong></td>
</tr>
</tbody>
</table>

**Empty**

**MMP13**
Implications for gene therapy in RA

- The disease-inducible promoters Saa3 and MMP13 are as effective as the constitutive PGK promoter for local expression of anti-inflammatory IL-10 and ameliorating SCW arthritis.

- In SCW arthritis, there is no need to overexpress IL-10 before onset of disease and can even be postponed to day 1 after disease onset as seen with the MMP13 promoter-vector.

- Disease regulated promoters can be used to temporal expression of biologics to enhance the therapeutic efficacy and limit side effects.
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