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

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No disclosures

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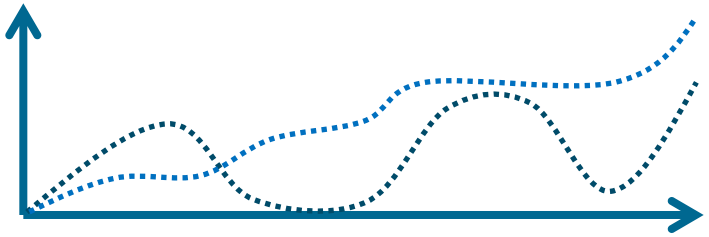
Rheumatoid Arthritis

• 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

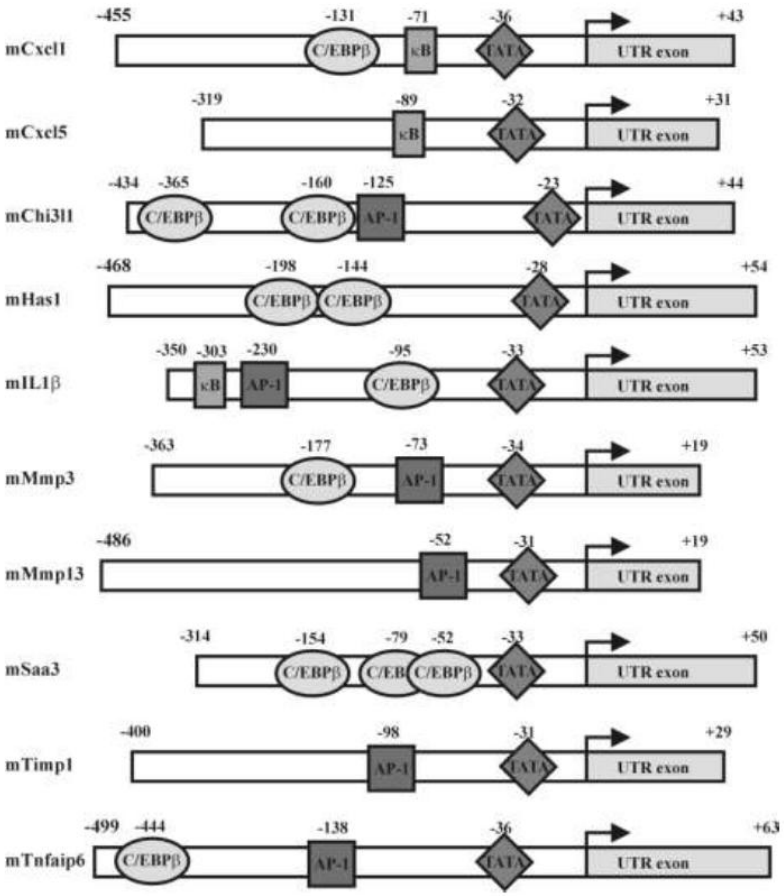
  • long-term treatment - even during remission

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

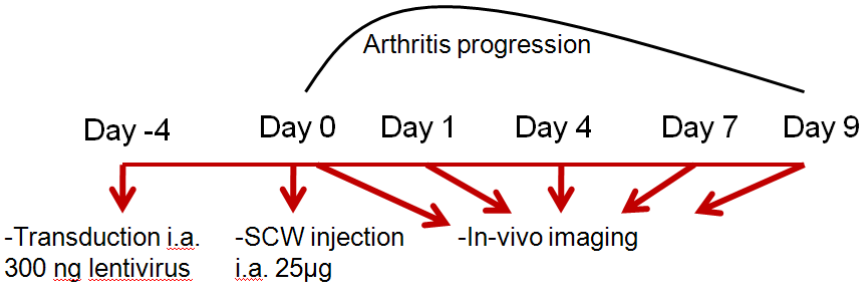


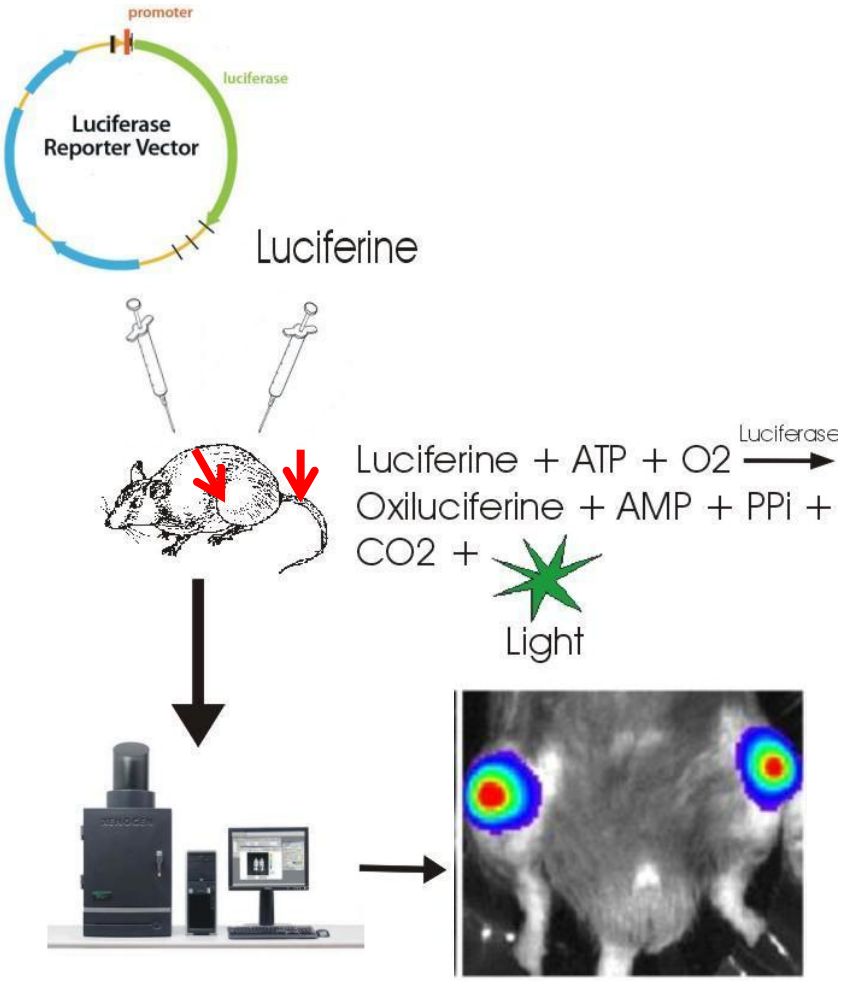
- Microarray of synovial tissue of mice with collagen induced arthritis
- Selection of genes upregulated during arthritis
- Prediction of regulatory elements on their transcriptional promoter
- Clone proximal promoter into viral expression vectors

Geurts *et al.* 2009

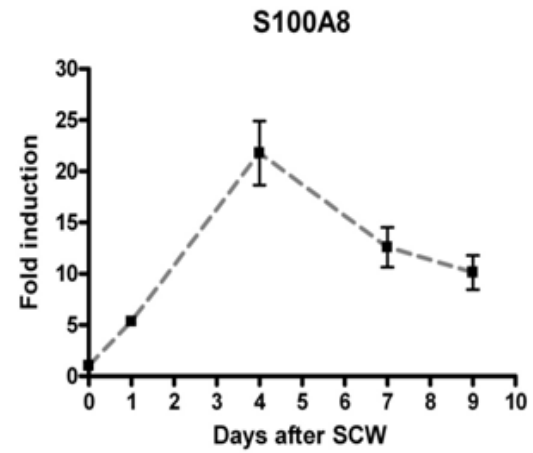
Validation: in-vivo profiling of selected promoters

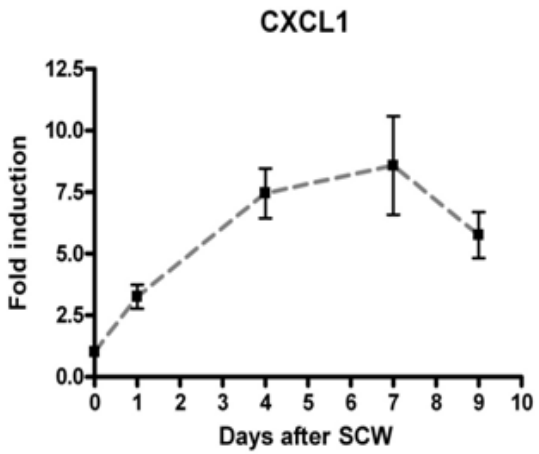
- 300 ng lentivirus intra-articular in knee joint
- Induction SCW arthritis 4 days after transduction.
- Imaging at day 0, 1, 4, 7 and 9

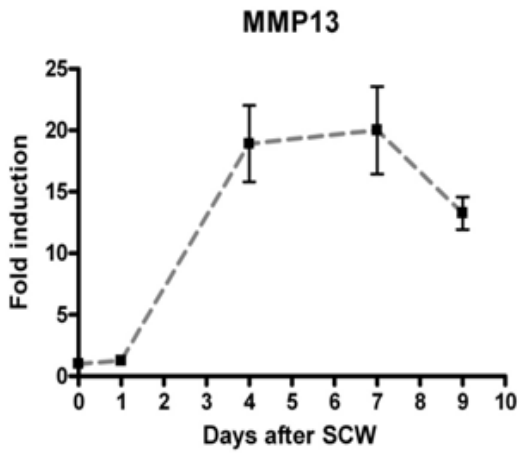


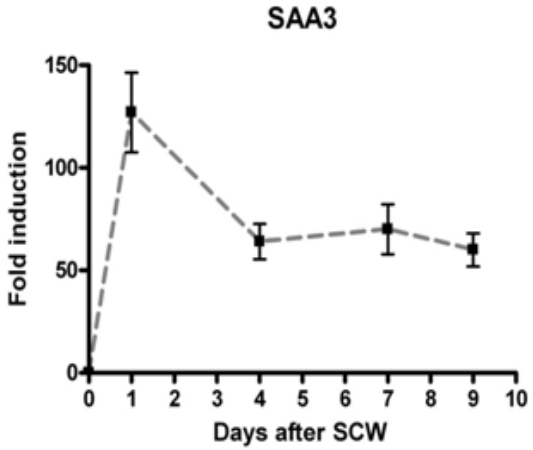


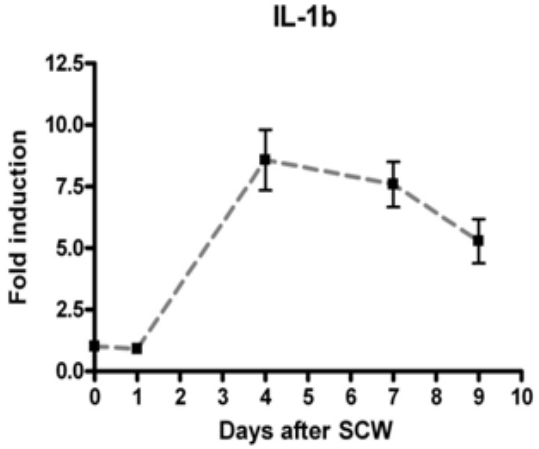
Kinetics of promoter-luciferase expression

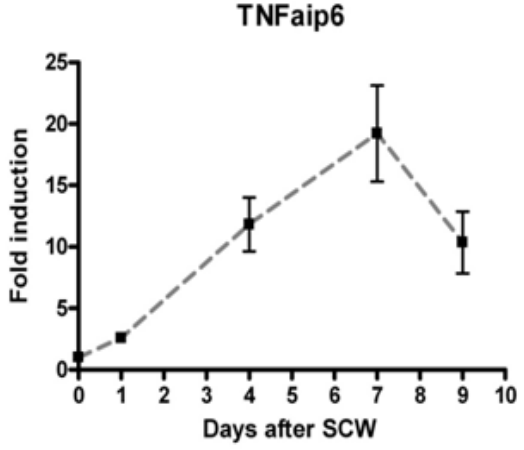








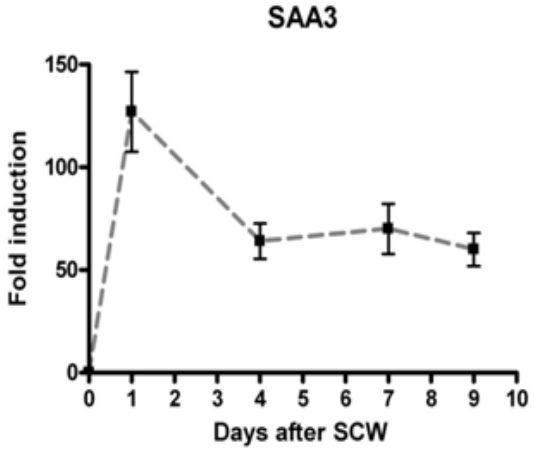


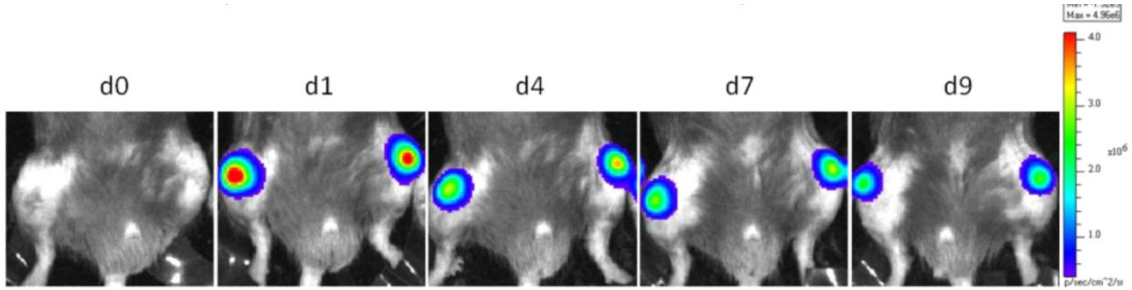


Promoter of serum amyloid A3 was selected

- 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

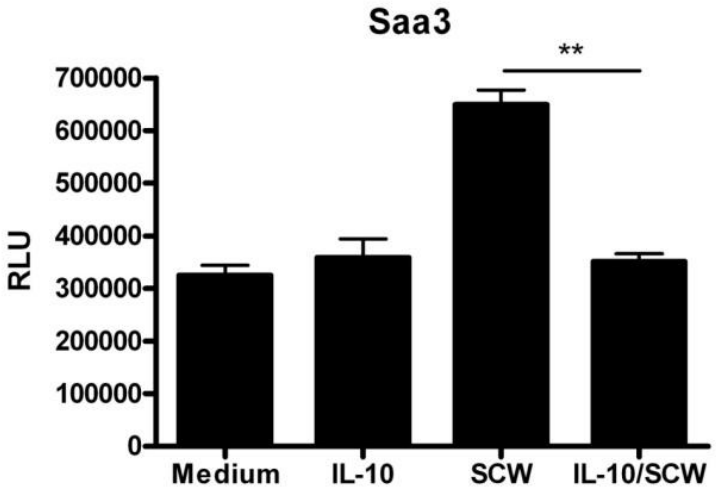
- Inhibits antigen-presentation (MHCII, costimulatory antigens)
- Capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF

- Can block NF-kB and STAT-activation
- SOCS3 and IL-1Ra

- Short half-life in serum: between 1.1 – 2.6 hours

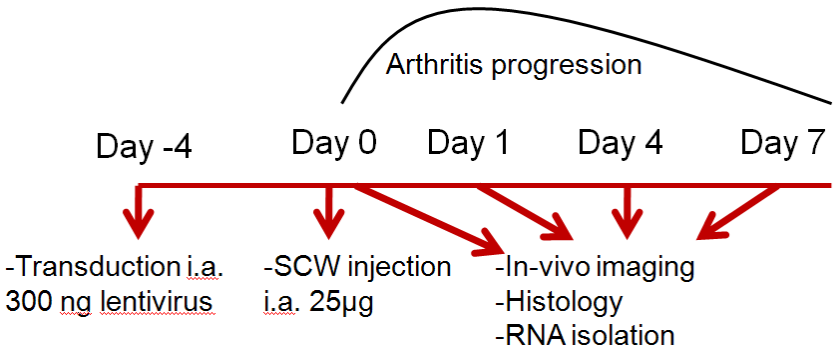
To prevent uncontrolled production the Saa3 promoter should not be activated by IL-10

- Stimulation of lentiviral transduced NIH-3T3 fibroblast cells
  - Transduced with LV.Saa3-Luc (50 ng p24^{gag} 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



Experimental setup arthritis experiment

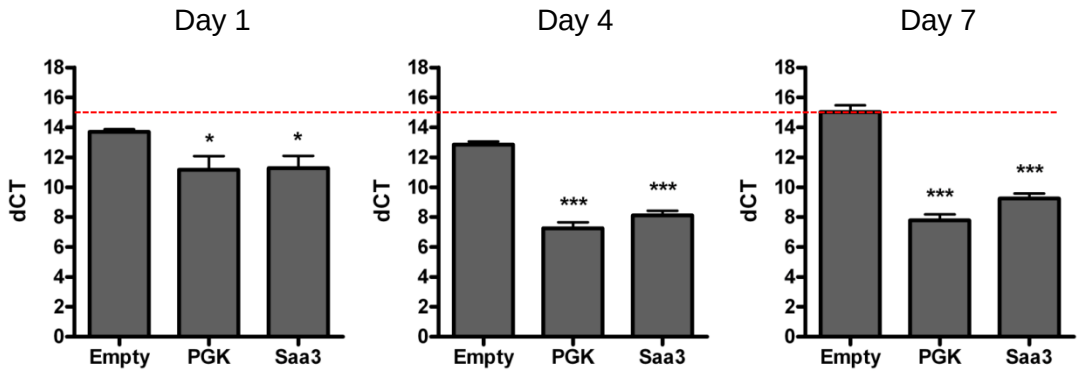
- Day -4 = i.a. injection lentivirus (300 ng p24)
  - PGK-Empty (virus control, Phosphoglycerate kinase promoter)
  - PGK-IL10 (positive control)
  - Saa3-IL10
- Day 0 = i.a. injection SCW (25µg)
- Day 1,4,7 = isolation knee joint / synovium for histology or RNA isolation + serum for cytokine analysis



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

PCR



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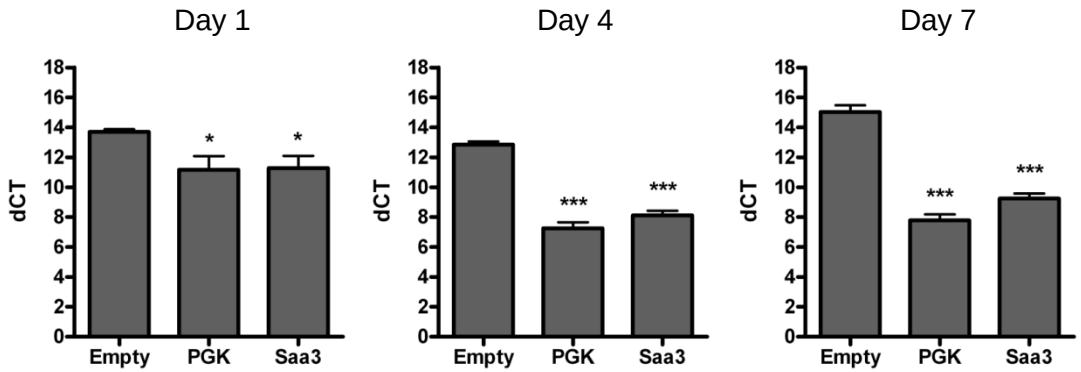
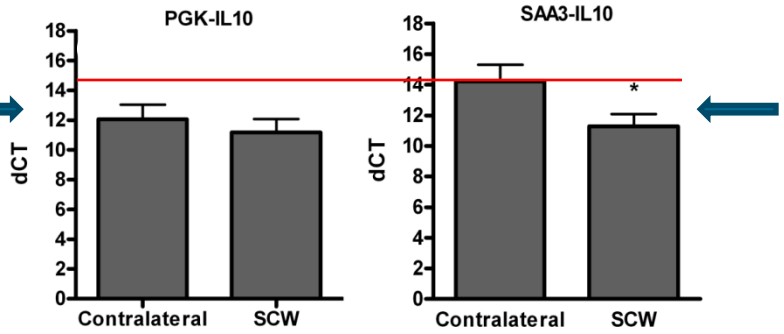


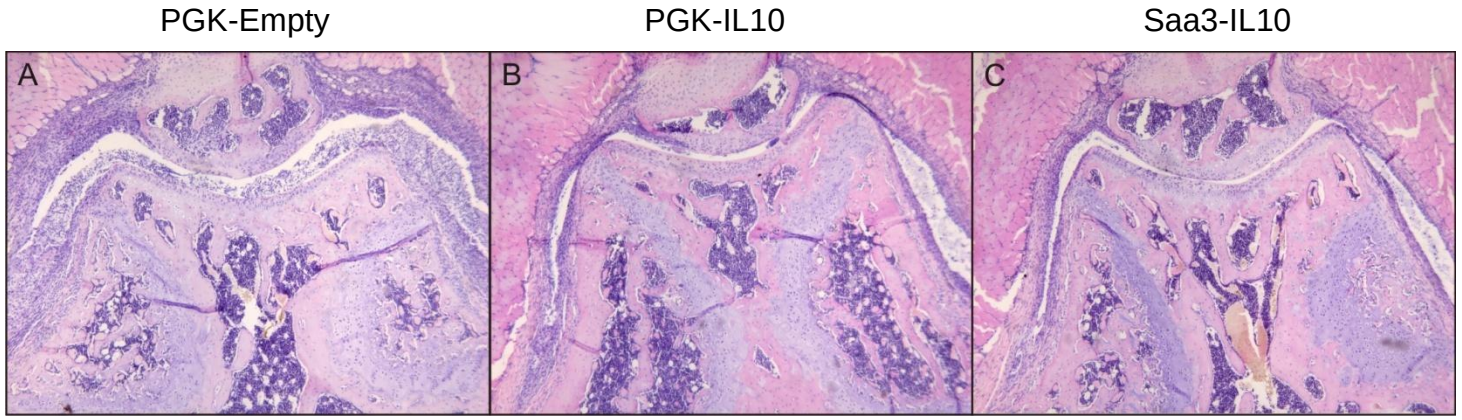
- Saa3 promoter shows selective and inducible response in the arthritic joint

Spontaneous production



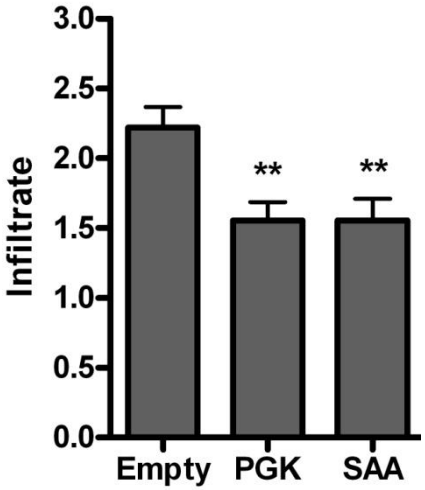
Inducible production

Histology at day 4

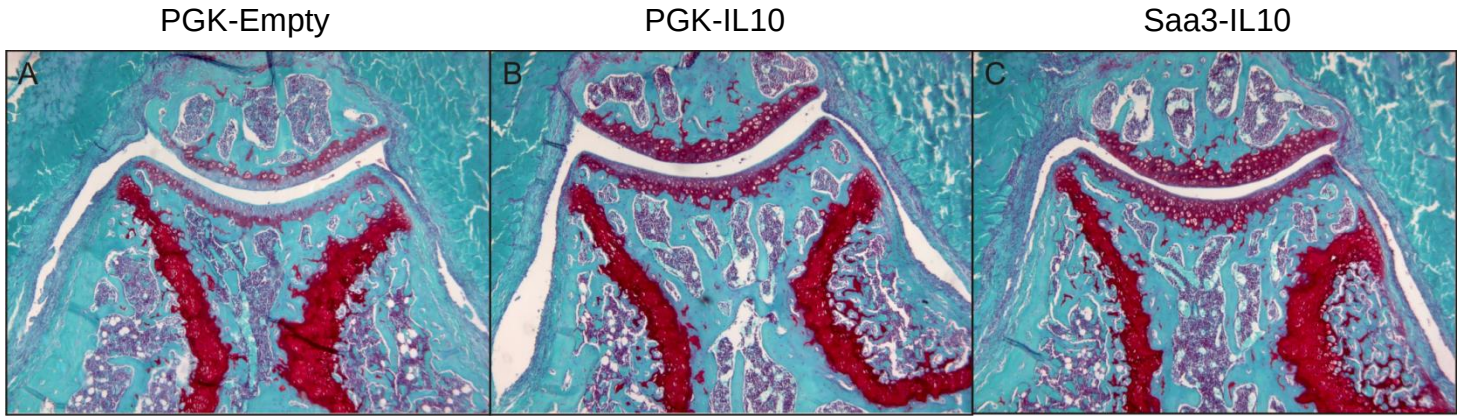


Day 4 after SCW

- Synovitis decreased at day 4

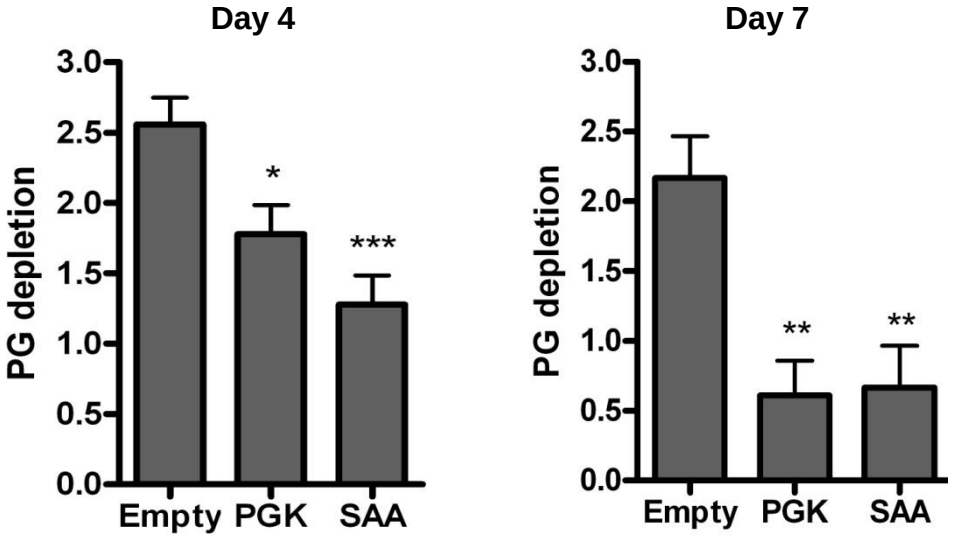


Cartilage damage at day 4 and 7



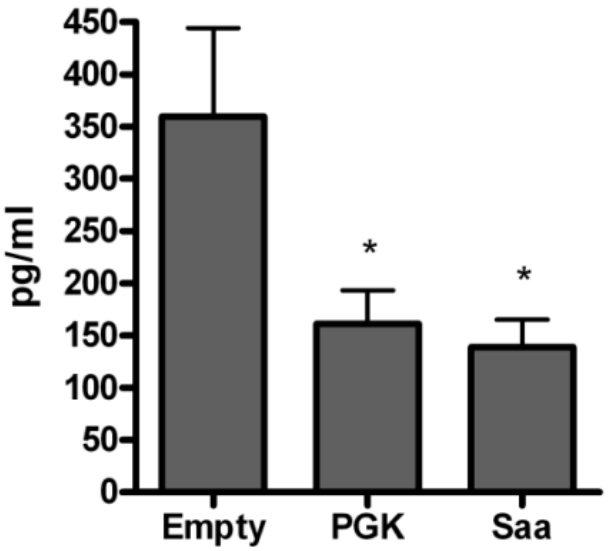
Day 7 after SCW

- Proteoglycan (PG) loss decreased at day 4 and 7

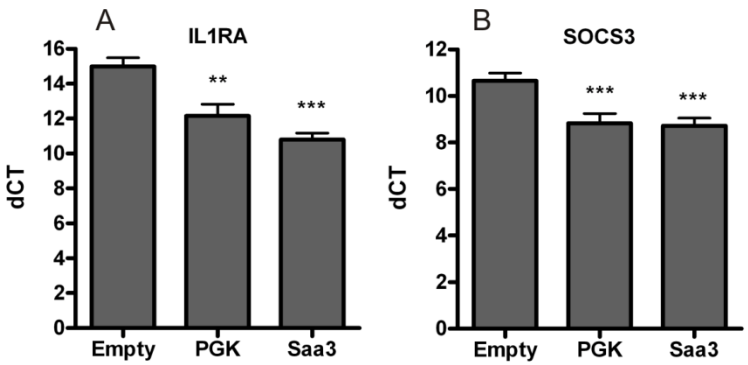


Effects of IL-10 overexpression on synovial cytokine production and gene expression

- 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



Day 7 after SCW

- 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!

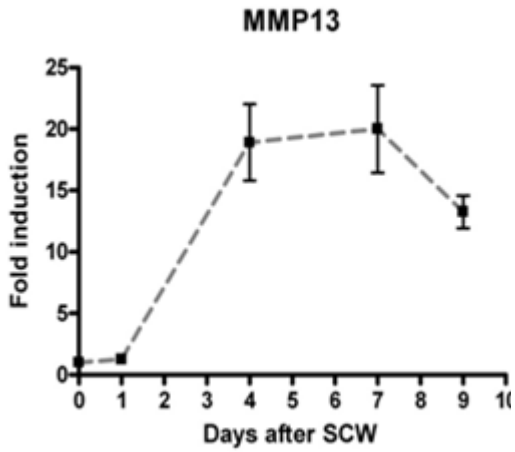
TABLE 1. Joint swelling, inhibition of cartilage PG synthesis and levels of cytokines during SCW arthritis

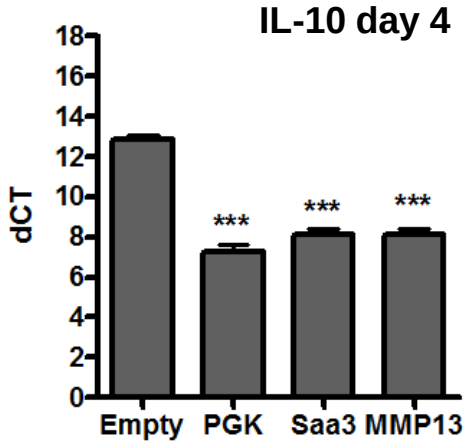
	Joint swelling (R/L ratio)	Inhibition of PG synthesis	IL-1β (pg/ml)	TNF-α (pg/ml)	mIL-10 (pg/ml)
1.5 h	ND	ND	100 ± 20	420 ± 50	<4
6 h	ND	−2 ± 4%	1190 ± 390	180 ± 40	23 ± 6
day 1	1.81 ± 0.11	−49 ± 6%	490 ± 120	<40	<4
day 2	1.49 ± 0.04	−43 ± 5%	150 ± 20	<40	<4
day 4	1.21 ± 0.09	−21 ± 4%	120 ± 17	<40	<4

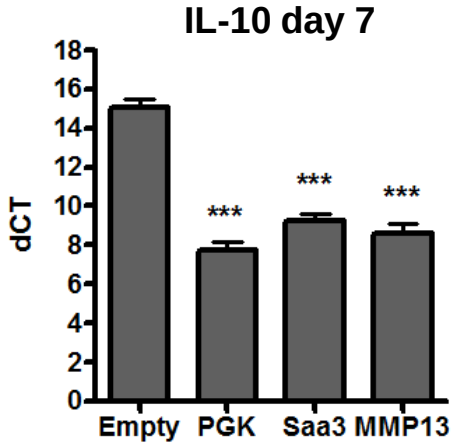
Unilateral arthritis was induced by intraarticular injection of 25 μg SCW into the right knee joint of naive mice. Joint inflammation was quantified by the ^{99m}Tc uptake method and the chondrocyte PG synthesis was assessed in patellae by $^{35}SO_4^{2-}$ incorporation ex vivo as described in Materials 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).

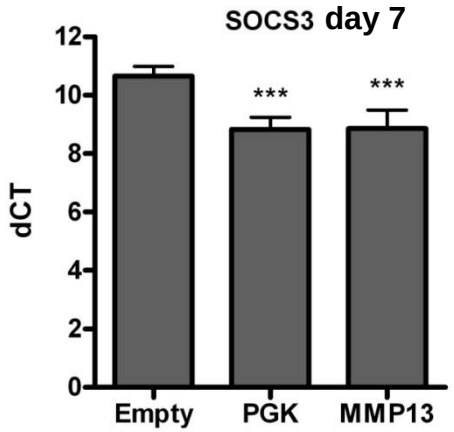
Lubberts et al. CYTOKINE, Vol. 10, No. 5 (May, 1998: 361–3)

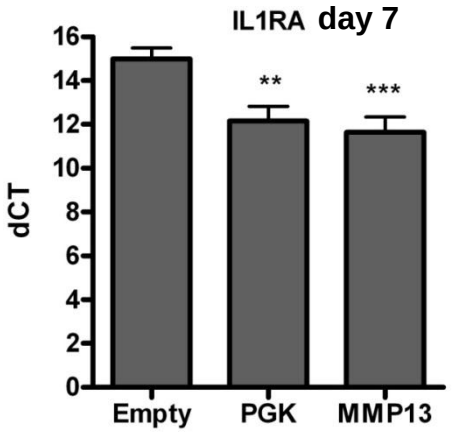
MMP13-IL10 could be as effective





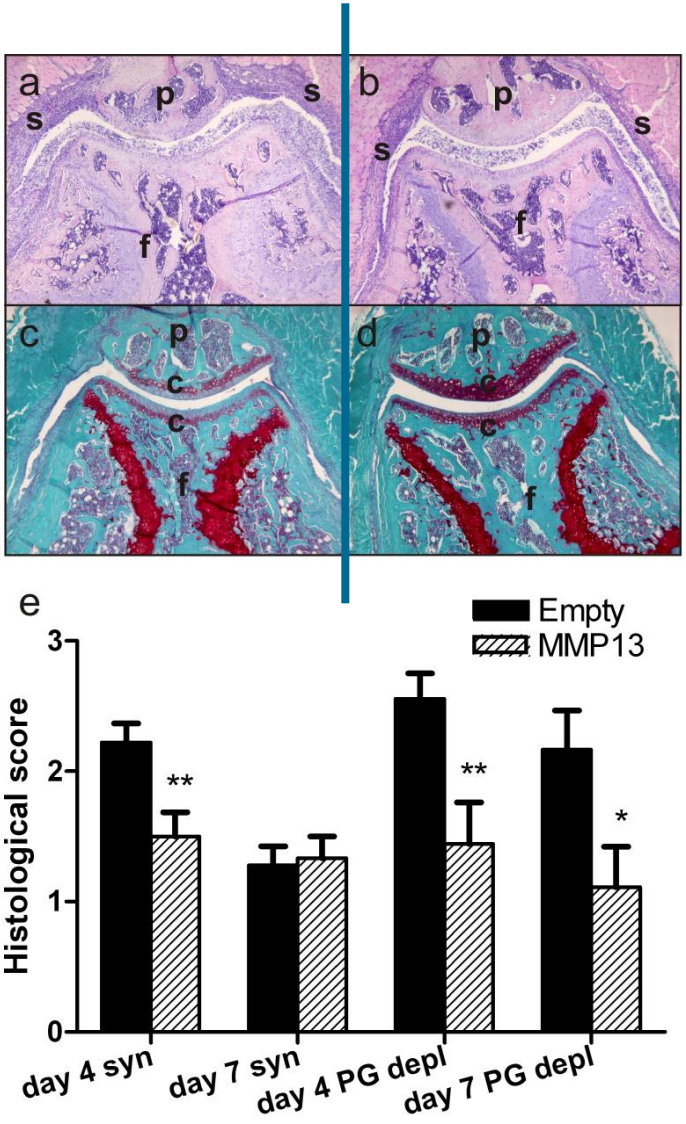






MMP13-IL10

SCW control virus



SCW MMP13-IL10 virus

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 been 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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SEVENTH FRAMEWORK PROGRAMME

NanoDiaRA

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