

## THE ROLE OF ACTIVATED SYNOVIAL MACROPHAGES IN OA

MACROPHAGE-PRODUCED CYTOKINES ARE INVOLVED IN DRIVING INFLAMMATORY AND DESTRUCTIVE RESPONSES IN OSTEOARTHRITIS

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Several authors have claimed that synovitis plays a role in the progression of osteoarthritis (OA). Overproduction of cytokines from the inflamed synovium can influence the production of degradative enzymes.

### QUESTIONS

1. Are there differences in macrophage function and signal transduction between RA and OA?
2. What is the role of the synovial macrophage in driving inflammatory and degradative responses in the OA synovium?
3. And what about the two main proinflammatory cytokines, TNF $\alpha$  and IL-1?

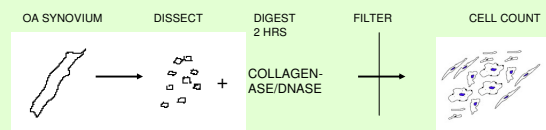
### 1. MACROPHAGE SIGNAL TRANSDUCTION IN OA

What role does the transcription factor NF $\kappa$ B play in the regulation of inflammatory and destructive mediators in the OA synovium?

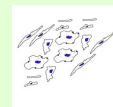
Are there differences between OA and RA?

We have used adenoviral gene transfer of I $\kappa$ B $\alpha$ , the endogenous inhibitor of NF $\kappa$ B, into OA synovial cells to investigate this.

### Preparation of primary OA cells



#### PRIMARY CELLS



- Adenovirus experiments
- Depletion experiments
- Inhibition experiments

### THE OA SYNOVIAL CELL COCULTURE MODEL

Fresh OA synovium is digested and the cells plated.

These cells are mainly synovial fibroblasts, 3-7% macrophages, less than 1% other cells.

These cocultures SPONTANEOUSLY secrete a variety of pro- and anti-inflammatory mediators as well as matrix metalloproteinases.

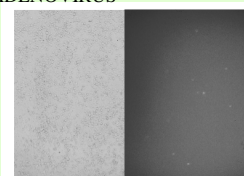
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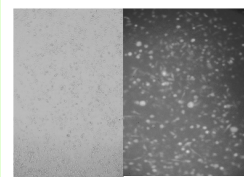
IL-6  
IL-8, MCP-1  
MMP-1,3,9,13  
TIMP-1

TNF $\alpha$   
IL-1 $\beta$   
Oncostatin M  
IL-10  
GM-CSF

### >95% OF OA SYNOVIAL CELLS CAN BE INFECTED WITH ADENOVIRUS

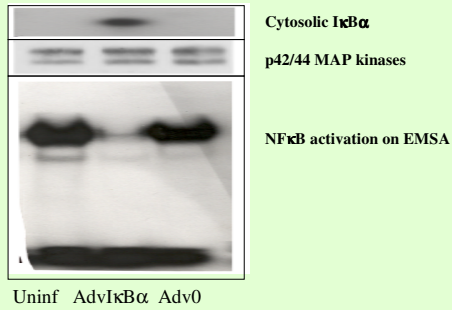


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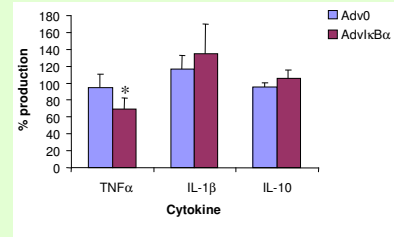


30:1 AdvGFP

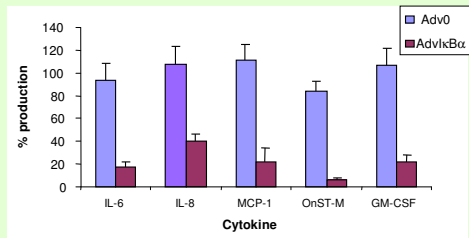
THE AdvIkB $\alpha$  ADENOVIRUS IS FUNCTIONAL IN OA SYNOVIAL CELLS



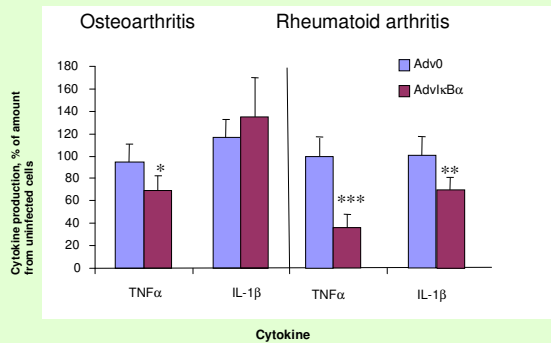
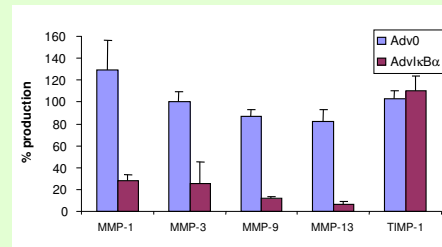
I $\kappa$ B $\alpha$  OVEREXPRESSION SLIGHTLY INHIBITS THE SPONTANEOUS PRODUCTION OF TNF $\alpha$  IN THE OA SYNOVIUM, BUT DOES NOT AFFECT IL-1 $\beta$  OR IL-10



EFFECT OF I $\kappa$ B $\alpha$  OVEREXPRESSION ON OTHER PROINFLAMMATORY CYTOKINES IN OA



I $\kappa$ B $\alpha$  OVEREXPRESSION INHIBITS THE SPONTANEOUS PRODUCTION OF SEVERAL MMPs IN THE OA SYNOVIUM, BUT NOT THEIR MAJOR INHIBITOR TIMP-1



CONCLUSIONS [MACROPHAGE FUNCTION]

- \* The OA synovium is a highly inflammatory environment, with production of many cytokines and MMPs
- \* Adenoviral gene transfer can be used to study signal transduction also in a mixed population of cells.
- \* The spontaneous production of many other proinflammatory cytokines, like IL-6, IL-8, MCP-1 and Oncostatin M, is NF $\kappa$ B dependent in OA, as is the production of several MMPs. This agrees with earlier findings using RA synovium.
- \* In contrast, the marked NF $\kappa$ B dependence of TNF $\alpha$  and IL-1 $\beta$  in RA cannot be observed in the OA synovium.

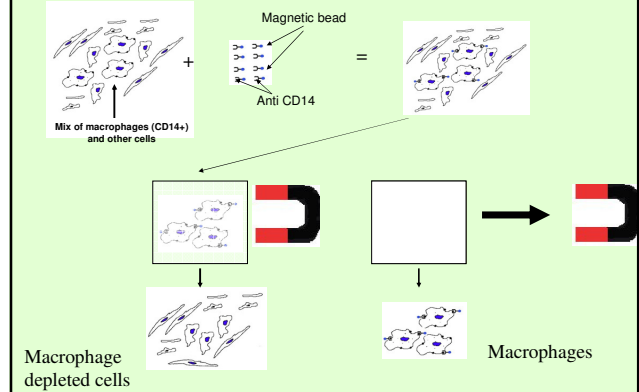
## 2. THE ROLE OF MACROPHAGES IN DRIVING INFLAMMATION AND DESTRUCTION IN OA

In the RA synovium, macrophage-produced  $TNF\alpha$  is a prime therapeutic target.

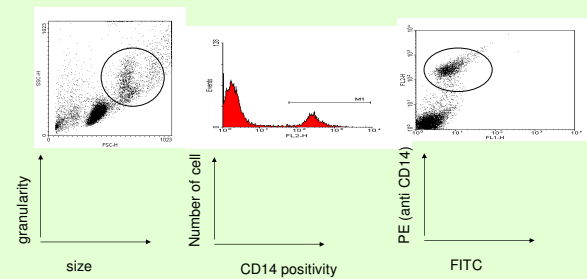
The OA macrophages are attracting increasing interest, due to various in vitro and in vivo observations.

Depletion of OA synovial macrophages might be a way to find out more...

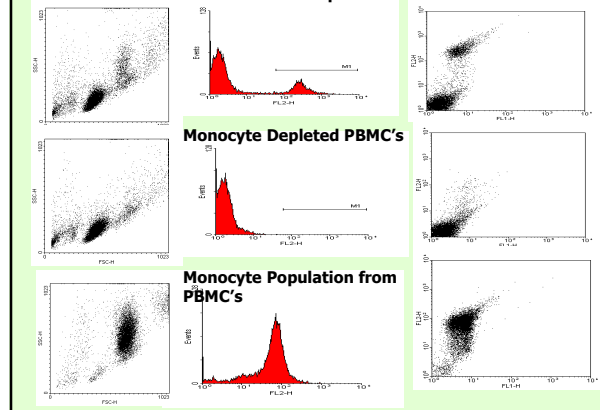
## Macrophage depletion using "MACS"



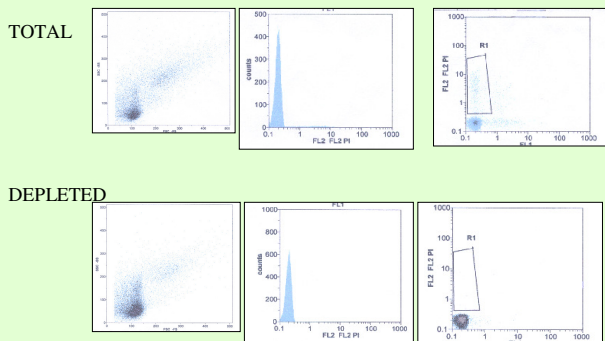
## FACS analysis for CD14 positivity in peripheral blood mononuclear cells



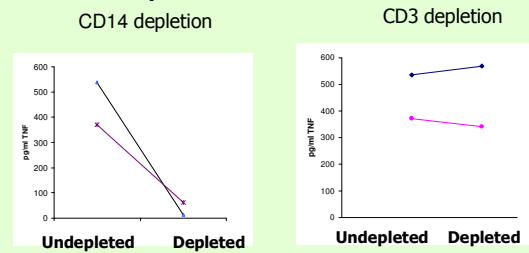
## Total PBMC Population



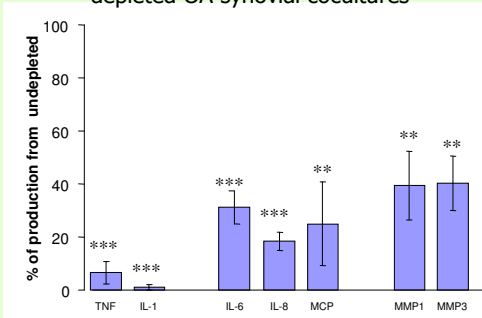
## FACS analysis of OA synovial cell cocultures



## Effect of macrophage (CD14) or T cell (CD3) depletion on the spontaneous $TNF\alpha$ production from OA synovial cell cocultures



**Cytokine and MMP production from macrophage depleted OA synovial cocultures**



**CONCLUSIONS [MACROPHAGES IN OA]**

It is technically possible to use macrophage depletion in mixed cell populations, including synovial cells.

Depletion of OA synovial macrophages results in marked downregulation of several fibroblast-produced cytokines and MMPs.

It appears as if the macrophages stimulate the synovial fibroblasts into producing cytokines like IL-6 and IL-8 and MMPs like MMP-1 and MMP-3.

**IF WE ASSUME THAT MACROPHAGES ARE IMPORTANT MOVERS IN OSTEOARTHRITIS:**

Do they stimulate other cells by direct contact, or through cytokines or other mediators?

Which macrophage-produced cytokines are the most important?

Is there a 'cytokine cascade' like there is in RA?

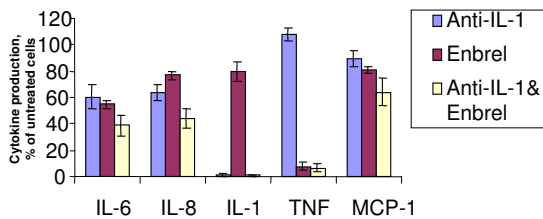
Can the macrophages affect the production of other proinflammatory cytokines from the synovial fibroblasts?

Can they influence the production of MMPs and aggrecanases?

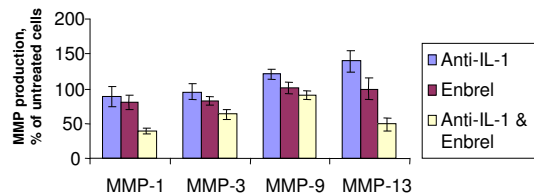
**3. IS THE OA SYNOVIAL MACROPHAGE EXERTING ITS EFFECTS ON CYTOKINES AND MMPS VIA TNF $\alpha$  AND/OR IL-1 $\beta$ ?**

- A coculture is produced by digesting OA synovium
- Cells are either left uninhibited, or Enbrel and/or anti-IL-1 $\beta$  is added
- Cells are incubated for 48 hours and the level of cytokines, MMPs and aggrecanases is tested by ELISA and RT-PCR

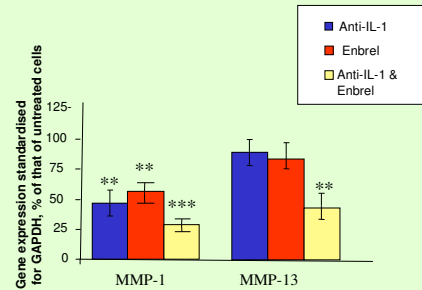
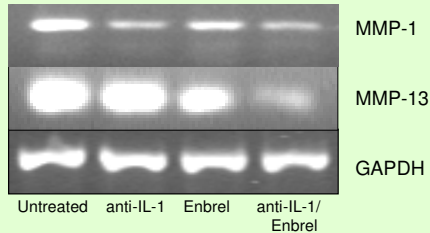
**The effect of anti-cytokine strategies on the production of pro-inflammatory cytokines in OA synovial cell cocultures**



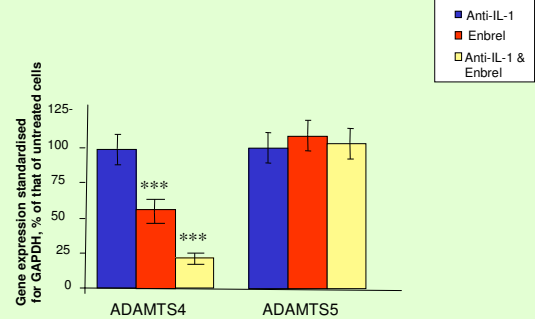
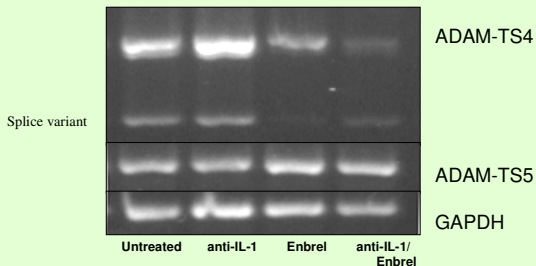
**Effect of anti-cytokine strategies on MMP production in OA synovial cocultures**



EFFECT OF ANTI-CYTOKINE TREATMENT ON COLLAGENASE EXPRESSION IN OA SYNOVIAL COCULTURES



EFFECT OF ANTI-CYTOKINE TREATMENT ON AGGREGANASE EXPRESSION IN OA SYNOVIAL COCULTURES



CONCLUSIONS [ANTI-CYTOKINE STRATEGIES]

In OA synovial cell cocultures, macrophages stimulate the synovial fibroblasts into producing cytokines and MMPs.

This effect is largely mediated via  $TNF\alpha$  and  $IL-1\beta$ , with neither cytokine playing a dominant role.

In contrast to the situation in RA, IL-1 is not TNF driven in the OA synovium.

ADAMTS4 is driven by  $TNF\alpha$  in the OA synovium, whereas ADAMTS5 is constitutive.

SOME RECENT PAPERS ABOUT MACROPHAGES AND CYTOKINES IN OA:

- \* Blom et al 2004: In murine experimental OA, depletion of synovial lining macrophages leads to impressive amelioration of osteophytes.
- \* Blom et al 2007: Depletion of synovial macrophages inhibits MMP-induced aggrecan cleavage. The macrophages appear to mediate MMP production in synovium rather than in cartilage.
- \* Grunke & Schulze-Koops 2006: A pilot study [only one patient] of Adalimumab for inflammatory OA of the knee was successful.
- \* Chevalier et al 2006: The IL-1ra had some analgesic effect in hand OA
- \* Magnano et al 2007: In an open-label study involving 12 patients, Adalimumab did not have a very potent effect on hand OA

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