IFN-γ OVEREXPRESSION ENHANCES CARTILAGE DESTRUCTION IN EXPERIMENTAL ARTHRITIS MEDIATED BY IMMUNE COMPLEXES

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Introduction
Immune complexes interact with synovial cells, infiltrating macrophages, and chondrocytes through Fcγ receptors on the surface of these cells. The type I and III receptors are activating receptors, which mediate release of inflammatory cytokines and destructive enzymes. Recently, we found that in an antigen induced arthritis (AIA), which is mediated by T-cells and immune complexes, FcγRI is the important activating FcγR. In contrast, during arthritis mediated solely by immune complexes (ICA), FcγRIII is dominant. Irreversible cartilage destruction is more pronounced in AIA as compared to ICA, implying that a T-cell factor enhances destruction, mediated by immune complexes. The cytokine interferon-γ (IFN-γ) is a major T cell derived mediator, which is known to upregulate the expression of FcγRI on macrophages.

In the present study we investigated whether overexpression of IFN-γ during experimental arthritis leads to more severe cartilage damage and if so, whether this effect is immune complex dependent. IFN-γ was overexpressed during an immune complex mediated arthritis (ICA) and zymosan arthritis (ZIA), which is not immune complex dependent. Inflammation and cartilage destruction were determined by histology.

Methods
An adenoviral IFN-γ vector was used to overexpress IFNγ in the knee joint of C57Bl/6 mice. As a control vector an adenoviral vector expressing GFP was used. The expression of IFN-γ after injection of PBS, 1 x 10⁷ pfu Ad-IFN-γ or Ad-GFP in naive knee joints was determined at different time points with ELISA. FcγRI mRNA expression was detected in samples of synovium taken from knee joints after injection of PBS, Ad-GFP, and Ad-IFN-γ. ICA was passively induced by α-lysozyme/PLL-Lysozyme complexes. Zymosan arthritis (ZIA) was induced by intra-articular injection of 180 µg zymosan particles. PBS, Ad-IFN-γ or Ad-GFP were injected one day prior to arthritis induction. Knee joints were dissected three days after intra-articular injection. H&E stained sections of total knee joints were used to study inflammation (inflammatory cell mass in joint cavity and synovial lining). Reversible cartilage destruction (proteoglycan depletion) was determined as the percentage of empty lacunae of the total amount of lacunae, whereas erosion was scored as the percentage ruffled cartilage surface of the total cartilage surface.

Results
After injection of PBS, Ad-IFN-γ or Ad-GFP, patella washouts were done at 6, 24, 48, and 72 hours and IFN-γ production was determined by ELISA. IFN-γ production after 6 hours was 1230 pg/ml and reached its detection level after 48 hours. Injection of Ad-GFP did not result in detectable production of IFN-γ. Overexpression of IFN-γ or GFP in naive knee joints did not induce inflammation or cartilage destruction. FcγRI mRNA levels were not detectable in naive knee joints before injection of Ad-IFN-γ. Uregulation of FcγRI mRNA was already found after 6 hours and sustained until day 7 after injection of Ad-IFN-γ. Injection of Ad-GFP and PBS did not result in upregulation of FcγRI. Injection of Ad-IFN-γ one day prior ICA induction, did not provoke inflammatory cell mass in the synovial lining (infiltrate) and joint cavity (exudate). Proteoglycan depletion was also similar in all groups. In contrast, chondrocyte death was increased 2- to 3-fold in the Ad-IFN-γ group compared to the PBS control group and even 4- to 6-fold compared to the Ad-GFP group (Figure 1). Due to the early time point erosion of the cartilage surface was absent in the control groups, whereas IFN-γ overexpression resulted in 10-25% erosion of the cartilage surface (Figure 2).

Discussion
In the present study we demonstrate that IFN-γ aggravates irreversible cartilage destruction in arthritis induced by immune complexes. An explanation for this aggravation might be stimulation of FcγRI on macrophages. Overexpression of IFN-γ increases the expression of FcγRI on the surface of macrophages and stimulation of FcγRI by ICs results in production of oxygen radicals and increased expression of destructive enzymes. An overburst of oxygen radicals may lead to chondrocyte death. Enzymes like collagenase, induce activation of matrix metalloproteinases (MMP) in the cartilage matrix, which eventually leads to erosion of the cartilage surface.