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Brief Report

Fc-gamma receptors are not involved in cartilage damage during experimental osteoarthritis

M. Stock †, *, A. Distler †, J. Distler †, C. Beyer †, G. Ruiz-Heiland †, N. Ipseiz †, M. Seeling ‡, G. Krönke †, F. Nimmerjahn ‡, G. Schett †

† Department of Internal Medicine 3, Erlangen Medical School, University of Erlangen–Nuremberg, Erlangen, Germany
‡ Department of Biology, Chair of Genetics, University of Erlangen-Nuremberg, Erlangen, Germany

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SUMMARY

Objective: Fc-gamma receptors (FcγRs) have been shown to play a crucial role in cartilage degradation during experimental arthritis. Although most of their effect on cartilage degradation has been attributed to their potential to promote inflammation in the presence of immunoglobulins, activating FcγRs promote cartilage degeneration in antigen-induced arthritis (AIA) independently of the level of inflammation. This prompted us to investigate, whether FcγRs may also play a role in osteoarthritis (OA)-related cartilage degradation.

Methods: FcγR expression was measured by RT-PCR and FACS in murine cartilage tissue and chondrocytes. Experimental OA was induced by destabilisation of the medial meniscus (DMM) in WT mice and animals lacking either activating (Fc receptor γ-chain-deficient) or inhibitory (FcγRIIB-deficient) FcγRs. Cartilage damage was investigated histologically 8 weeks post-surgery by assessing proteoglycan loss and structural damage according to OARSI recommendations. Osteophyte size was measured to investigate alterations in bone turnover.

Results: Expression analyses revealed significant levels for all four types of murine FcγRs in mouse chondrocytes and cartilage tissue from newborn and 8-week-old mice. Surprisingly, yet, ablation of either activating or inhibitory FcγRs did not affect cartilage damage or bone turnover during DMM-induced OA in mice.

Conclusion: While FcγRs appear to have a crucial role in cartilage degradation during inflammatory arthritis our data indicate that FcγRs do not influence cartilage destruction in experimental OA. This indicates that a certain threshold of inflammation is a prerequisite for FcγR-induced cartilage destruction in arthritis.

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Introduction

Articular cartilage degradation is one of the primary hallmarks of both inflammatory and degenerative joint diseases and prominently contributes to their disease burden. In contrast to bone and other mesenchymal tissues, cartilage does not sufficiently regenerate after injury, even if the trigger for cartilage loss can be controlled by clinical intervention. Therefore, the investigation of the pathological molecular events that disturb the balance of cartilage turnover is a prerequisite for future therapies that aim to restore cartilage homeostasis.

In inflammatory arthritis, resident fibroblasts and macrophages secrete large amounts of catabolic enzymes, particularly aggrecanases of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family and collagenases of the matrix metalloproteinase (MMP) family, which degrade the cartilage matrix, ultimately destroying the articular cartilage[1]. In osteoarthritis (OA), cartilage breakdown results from a dysbalance of cartilage matrix synthesis and degradation. Thus, corrupt TGFβ signalling

* Address correspondence and reprint requests to: M. Stock, Department of Internal Medicine 3, University of Erlangen-Nuremberg, 91054 Erlangen, Germany. Tel: 49-9131-8529101; Fax: 49-9131-8526341.

E-mail addresses: mstock@molmed.uni-erlangen.de (M. Stock), alfiya.distler@uk-erlangen.de (A. Distler), joerg.distler@uk-erlangen.de (J. Distler), christian.beyer@uk-erlangen.de (C. Beyer), giselaruizh@yahoo.com.ar (G. Ruiz-Heiland), natacha.ipseiz@uk-erlangen.de (N. Ipseiz), michaela.seeling@fau.de (M. Seeling), gerhard.kroenke@uk-erlangen.de (G. Krönke), falk.nimmerjahn@fau.de (F. Nimmerjahn), georg.schett@uk-erlangen.de (G. Schett).

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and induction of transcription factors such as Hif2α increase activity of ADAMTS and MMPs in chondrocytes[2]. Together with a decrease in the production of cartilage matrix proteins such as aggrecan and collagen type II, a dysbalance in cartilage turnover occurs which leads to cartilage loss in OA[2,3]. Although inflammatory arthritis and OA are very different pathologies, the terminal pathway leading to cartilage destruction appears to involve common mechanisms with ADAMTS- and MMP-mediated degradation of the cartilage matrix.

Previous studies have shown that the absence of activating Fcγ receptors (FcγRI, FcγRIII, and FcγRIV) abrogated cartilage destruction during experimental arthritis, indicating a critical role of FcγR signalling in the process of cartilage destruction in arthritis[4,5]. This has been mostly attributed to the role of FcγRs in immunoglobulin-dependent activation of macrophages and neutrophils during inflammation[6,7]. However, in antigen-induced arthritis (AIA), loss of activating Fcγ receptors has been shown to uncouple inflammation and cartilage destruction[4]. Together with the finding that FcγRIIB and III are expressed in chondrocytes this may point to a direct role of FcγR signalling in chondrocyte activity and cartilage degradation[8].

In this study, we demonstrate that all four murine types of FcγRs are present in mouse chondrocytes. Therefore, we hypothesised that FcγR signalling may also play a role in the progression of OA. In order to test this hypothesis we surgically induced experimental OA in wild-type animals, in mice devoid of the Fc receptor γ-chain (and therefore deficient in activating FcγRI, FcγRIII and FcγRIV), and in FcγRIIB-deficient mice and investigated OA-dependent cartilage damage. Surprisingly, however, the lack of FcγR did not alter the severity of cartilage destruction in experimental OA.

Methods

Cells

Primary murine chondrocytes were isolated from epiphyses or rib cages of newborn mice (aged 3–5 days) by consecutive enzymatic digestion with trypsin and collagenase P as previously reported[9]. To minimise phenotypical changes chondrocytes were cultured in monolayers for no longer than 7 days with a maximum of one passage. Macrophages were isolated from bone marrow by flushing the femurs and tibias of 8–10 week-old C57BL/6 WT mice. The next day, non-adherent cells were collected and cultured in α-MEM medium containing 10% FCS, 1% Penicillin/Streptomycin and 30 ng/ml of M-CSF (R&D) for 7 days.

Gene expression analysis

For gene expression analyses RNA was extracted from epiphyseal cartilage dissected from newborn mice or from articular cartilage dissected from the femoral heads of 8-week-old mice or from cultured cells using the RNeasy (Fibrous Tissue for tissue) Kit (Qiagen) according to the manufacturer's instructions. RT-PCR for the detection of collagen2a1 (Col2a1) and cyclophilin A was carried out as previously reported using the Superscript II reverse transcriptase system (Invitrogen) and the TaqPCR Core Kit (Qiagen)[10]. Additional gene-specific primers: FcγRI: 5′-TTAAGCGCAGCCCTGAGT-3′ (forward), 5′-TCCCACTGACAGATAAAACAGG-3′ (reverse); FcγRIIB: 5′-AAAGCAGGTTCCAGACAATCC-3′ (forward), 5′-GATGCTTGAGAAGTG AGTAGGTGAT-3′ (reverse); FcγRIII: 5′-CAAGCCTGTCACCATCACTG-3′ (forward), 5′-GAGGCACATCACTAGGGAGAA-3′ (reverse); FcγRIV: 5′-GGGCTCATTGGACACAACA-3′ (forward), ATGGATGGAGACCCTGGAT (reverse). CD45: 5′-CCTCTGGAGGCTGAATACCA-3′ (forward), 5′-TGCTCATCTCCAGTTCATGC-3′ (reverse).

FACS analysis

Rib chondrocytes from newborn WT or FcγRIIB-/FcRγ-chain-double-deficient mice were trypsinised and passed through a cell strainer. After blocking with Fc block (2.4G2 or 9E9 respectively, both self-produced) cells were stained with an antibody mixture containing DAPI, APC-Cy7-labelled CD45 (clone 30-F11; BioLegend), Pe-Cy7-labelled CD31 (clone 390; BioLegend) and either APC-labelled FcγRI (CD64; clone X54-5/7.1, BD Biosciences), FcγRIIB (clone Ly17.2, self-produced), FcγRIII (CD16; clone 275003, R&D Systems) or FcγRIV (clone 9E9, self-produced). Data acquisition and analysis was performed with FACS Diva software (BD Biosciences). Chondrocytes were gated as alive SSChigh, CD45- and CD31- cells after exclusion of doublets.

Mice and induction and scoring of experimental OA

C57BL/6 mice were obtained from Elevage Janvier. FcγRIIB-deficient (generated on C57BL/6 background), FcRγ-chain-deficient and FcγRIIB-/FcRγ-chain-double-deficient mice (both backcrossed onto C57BL/6 background for at least 13 generations) were provided by Jeffrey Ravetch (The Rockefeller University, New York). Phenotypical abnormalities of the musculoskeletal system have not been described for untreated mutant mice used in this study, except for some minor reduction of osteoclast counts in FcRγ-chain-deficient mice, which did not affect bone homeostasis[11]. We could not observe any significant abnormalities in growth, fertility, development or morphology of the musculoskeletal system of the mutant mice. Mutant mice were indistinguishable in size and weight from WT C57/B6 mice. Experimental OA was induced by destabilisation of the medial meniscus (DMM) through surgical transection of the medial meniscotibial ligament in male mice at the age of 8 weeks. Sham surgery was performed in the same way, including anaesthesia and opening of the knee capsule, but without transecting the ligament. DMM and sham operations were carried out in separate groups with both legs undergoing surgery. Eight weeks post-surgery mice were sacrificed and frontal paraffin sections (4 μm) of the knees were prepared and stained with Safranin-O. Proteoglycan-loss was quantified by determining the proportion of Safranin-O staining-negative articular cartilage using ImageJ software. OA scoring was performed in a blinded manner according to OARSI recommendations, scoring all four quadrants (medial and lateral femoral condyles, medial and lateral tibial plateaus) in multiple step sections through the joint[12]. Maximal scores from all four quadrants were cumulated. Osteophyte size at the medial tibia was measured on Safranin-O stained sections using ImageJ software. $n = 6$ per group. All animals were maintained under specific pathogen-free conditions. All experiments were performed with the approval of the local ethics authorities (Tierschutzbeauftragter of the University of Erlangen-Nuremberg and the Government of Mittelfranken, Ansbach, Germany) and according to the rules and regulations of the animal facilities in Germany and the United States.

Statistical analysis

Data are presented as the mean ± 95% CI. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and Tukey's multiple comparison test after confirming normal distribution using the Kolmogorov–Smirnov test. Additionally, significance was evaluated using the Mann–Whitney test. Statistical analysis was performed using Graph-Pad Prism software.

Results

Fcγ receptors are expressed in murine chondrocytes

In order to investigate, which types of FcγRs are expressed in cartilage, gene expression of all four murine types of FcγR was analysed by RT-PCR and compared with their expression in bone marrow macrophages. Substantial collagen2a1 expression confirmed the chondrocyte phenotype, and lack of CD45 expression excluded a contamination of cartilage tissue and primary chondrocyte cultures with haematopoietic cells. As expected these analyses confirmed the expression of FcγR types IIB and III in epiphyseal cartilage, and in chondrocytes from epiphyses and ribs from newborn mice. Moreover, cartilage and chondrocytes also exhibited significant expression levels of FcγR types I and IV (except rib chondrocytes) [Fig. 1(A)]. Furthermore, FcγR expression remained stable in articular cartilage from adult mice at the age of 8 weeks as compared to epiphyseal cartilage from newborn mice [Fig. 1(A)]. Finally, specific cell surface expression could be demonstrated for FcγRI in 4 of 4 experiments, and for FcγRIIB and FcγRIII in 3 of 4 experiments using FACS analysis. FcγRIV could only be detected in 2 of 4 experiments. A typical experiment for FcγRI-III protein expression is shown in Fig. 1(B).

These results indicate significant expression of Fcγ receptors in cartilage and may point to a direct role for FcγR signalling in chondrocyte biology.

Loss of activating or inhibitory Fcγ receptors does not alter development of experimental OA

Since FcγR signalling is involved in cartilage degradation during inflammatory arthritis[4] we assessed the effect of FcγR ablation in an experimental OA model. Therefore, we induced OA in 8-week-old FcR-γ-chain-deficient (devoid of all activating FcγRs), FcγRIIB-deficient, and wild-type C57BL/6 mice. Eight weeks post-surgery OA-related cartilage damage was investigated histologically by quantifying proteoglycan loss as Safranin-O-staining negative cartilage [Fig. 2(A) and (B)] and structural cartilage damage using the OARSI score for mouse OA [Fig. 2(A) and (C)]. While sham-operated control mice of all groups investigated did not exhibit significant or different levels of proteoglycan loss ($P = 0.65$) or cartilage destruction ($P = 0.74$), all mice that had undergone DMM surgery developed a significant (all $P < 0.001$, when compared to sham controls) degree of OA-mediated proteoglycan loss and structural cartilage damage. Surprisingly, however, there was no significant difference in proteoglycan loss ($P = 0.63$) or structural changes ($P = 0.98$) observed in WT mice and mice deficient in either activating or inhibitory FcγRs. Similar to cartilage destruction, osteophyte formation was barely detectable and not significantly different in sham-operated mice of either genotype ($P = 0.16$). In contrast, significant development of osteophytes (all $P < 0.001$, when compared to sham controls) was observed in either WT or FcγR mutant strains after DMM surgery. Osteophyte size after DMM surgery, however, did not significantly differ between WT, FcγRIIB- and FcR γ-chain-deficient mice ($P = 0.90$) [Fig. 2(D)]. One-way ANOVA to test for statistical significance shown here was confirmed using Mann–Whitney tests (Suppl. Table 1). These data indicate that FcγRs are not involved in cartilage degeneration or bone remodelling during experimental OA.

Discussion

This study aimed to investigate a potential direct effect of FcγR signalling on cartilage destruction during experimental OA. Therefore, we studied FcγR expression in murine chondrocytes and found all four types of FcγR being significantly expressed. Surprisingly, however, histological evaluation of cartilage damage in mice lacking either activating or inhibitory FcγRs revealed no difference in the OA-associated cartilage changes compared to Fcγ-receptor expressing wild-type controls.

Recent studies have put emphasis on the prominent role of FcγR signalling in the process of cartilage degradation during inflammatory arthritis. Immune complexes containing autoantibodies, which trigger FcγR signalling, are abundantly present in articular cartilage during arthritis. OA cartilage samples also show elevated levels of immune complexes[13]. FcγR signalling has been shown to be particular important for macrophage activation. Macrophage activation in turn has been demonstrated to be a key factor in cartilage degeneration during inflammatory arthritis[4,14]. In contrast, in the AIA model of inflammatory arthritis, Lent and colleagues found that activating FcγRs are necessary for arthritis-triggered cartilage destruction, independently of the level of

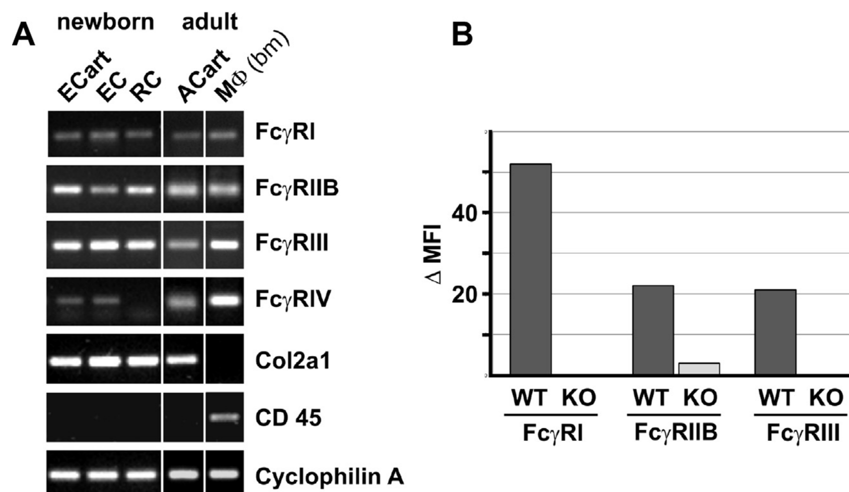

Fig. 1. FcγRs are expressed in murine chondrocytes. A: Total RNA was extracted from murine chondrocytes, newborn epiphyseal cartilage, adult (8 weeks) articular cartilage and macrophages. RT-PCR analyses were performed to detect mRNA levels of FcγR types I, IIB, III, and IV, collagen2a1 (Col2a1) and CD45. Cyclophilin A was used as a reference gene. ECart: epiphyseal cartilage; EC: epiphyseal chondrocytes; RC rib chondrocytes; ACart: articular cartilage; MΦ (bm): bone marrow macrophages. B: Rib chondrocytes from WT or FcγRIIB-/FcRγ-chain-double-deficient (KO) mice were analysed for FcγR cell surface expression using flow cytometry. Shown is the delta median fluorescence intensity (Δ MFI) compared with unstained samples of a typical experiment. All experiments shown were performed at least three times with comparable results.

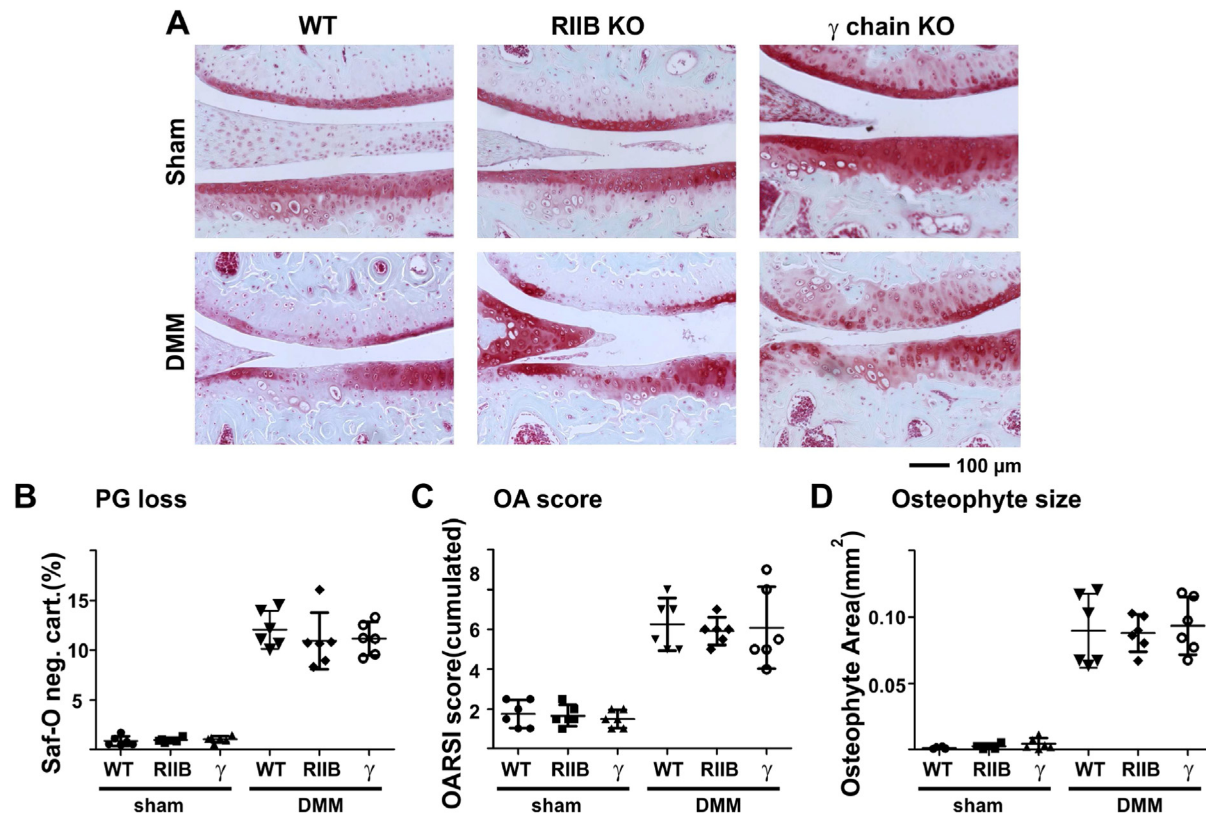

Fig. 2. Deficiency in FcγRs does not alter cartilage damage in DMM-induced OA. DMM or sham surgery was performed in WT, FcγRIIB-deficient (RIIB), or FcR γ-chain-deficient (γ) mice. Eight weeks post-surgery, cartilage damage and osteophyte development was investigated histologically. A: Safranin-O staining of frontal knee sections showing the medial part of the stifle joint. B: Quantification of proteoglycan (PG) loss, determined as the fraction of Safranin-O staining negative articular cartilage (Saf-O neg. cart.). C: Scoring of OA-induced cartilage damage according to OARSI recommendations. Maximal scorings of all four quadrants of the joint were cumulated per mouse. D: Osteophyte development was determined by measuring the size of osteophytes at the medial tibiae. Mean values ± 95% CI are shown. $n = 6$.

inflammation. They discussed that FcγR-dependent activation of synovial macrophages may trigger these cells to secrete catabolic mediators, such as oxygen radicals, TNFα and MMPs, which in turn mediate cartilage destruction through chondrocyte death and matrix degeneration[4].

In this study, we provide evidence for significant expression of all four types of murine FcγRs in newborn and adult mouse cartilage. At least for FcγR types I–III we could also provide evidence for cell surface expression on chondrocytes. These findings encouraged us to investigate, whether FcγR signalling in chondrocytes may serve as an alternative or additional pathway, how FcγRs might contribute to cartilage destruction during joint diseases. In order to test this hypothesis we investigated cartilage degeneration in the DMM mouse model for OA, which is known to develop only marginal levels of inflammation and macrophage invasion[15]. Surprisingly, however, we found that cartilage degradation, as well as osteophyte formation, was independent of the presence of either activating or inhibitory FcγRs in the DMM model.

These data indicate that FcγRs are not involved in the process of cartilage destruction or bone remodelling during DMM-induced OA in mice. Hence, FcγR-dependent cartilage degradation may rely on an inflammatory background, present in murine and human inflammatory arthritis. The role of FcγR expression and cell surface expression in chondrocytes remains to be elucidated.

Author contributions

Conception and design: MS, JD, CB, GR-H, GK, FN, GS. Data analysis and interpretation: MS, AD, NI, MSe. Drafting and revising manuscript: MS, AD, JD, CB, GR-H, NI, MSe, GK, FN, GS. Approving final version of manuscript: MS, AD, JD, CB, GR-H, NI, MSe, GK, FN, GS.

MS takes responsibility for the integrity of the data analysis.

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Competing interest statement

There are no conflicts of interests or support from commercial sources.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.02.019.

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