Immune regulation by receptors for IgG

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Chapter 5

The inhibitory receptor FcγRII reduces joint inflammation and destruction in experimental immune complex-mediated arthritides not only by inhibition of FcγRI/III but also by efficient clearance and endocytosis of immune complexes

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The Inhibitory Receptor FcγRII Reduces Joint Inflammation and Destruction in Experimental Immune Complex-Mediated Arthritides Not Only by Inhibition of FcγRI/III but Also by Efficient Clearance and Endocytosis of Immune Complexes

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Studies of FcγRII−/− mice identified the inhibitory function of this receptor in joint inflammation and cartilage destruction induced with immune complexes (ICs). To extend our insight in the role of FcγRII in arthritis, we explored the role of FcγRII in the absence of activating receptors I and III using FcγRI/III−/− as well as FcγRI/II/III−/− mice. When antigen-induced arthritis (AIA) was elicited, which is a mixture of T cell and IC-driven inflammation, arthritis was almost absent at day 7 in FcγRI/III−/− mice. Remarkably, in FcγRI/II/III−/− mice, this model induced a tremendously increased arthritis as compared to wild-type controls. This implies that FcγRII regulates joint inflammation also in the absence of activating FcγRI and III. To confirm the IC specificity of this finding, similar studies were done with ICs or zymosan as arthritogenic stimuli. Strongly elevated inflammation was found in FcγRI/II/III−/− mice with IC but not with zymosan. Clearance studies identified accumulation of IgG in the knee joint in the absence of FcγRII. Moreover, macrophages expressing only FcγRII showed prominent endocytosis of preformed soluble ICs not different from controls. In total absence of FcγR (FcγRI/II/III−/−), macrophages completely failed to endocytose ICs. Although joint inflammation was much higher in AIA arthritic knee joints of FcγRI/II/III−/− and the inflammatory cells still expressed an inflammatory phenotype, severe cartilage destruction (MMP-mediated neoepitopes in the matrix and chondrocyte death) was completely prevented in contrast to the marked destruction which was observed in the wild-type. Our study indicates that FcγRII reduces joint inflammation in the absence of activating FcγR by promoting endocytosis and clearance of ICs from the joint. Infiltrating cells, which fail to express activating FcγR although they still become stimulated are no longer capable of inducing severe cartilage destruction. *(Am J Pathol 2003, 163:1839–1848)*

Rheumatoid arthritis (RA) is a heterogenous chronic joint disease characterized by invasion of leukocytes and local synovium activation, which leads to severe destruction of cartilage and bone.[1] The most prominent leukocyte present within the inflamed synovium is the macrophage. A strong correlation was found between the number of activated macrophages and severe cartilage destruction.[2] In a normal joint, macrophages are comprised within the intima layer which covers the surface of the synovium.[3] In RA, synovial macrophages become activated, resulting in the release of chemokines, cytokines, and enzymes involved in regulation of joint inflammation and cartilage/bone destruction.[4,5]

The mechanism by which synovial intima macrophages become activated during RA is not known. One of the potential candidates are IgG-containing ICs. They are abundantly found in RA synovial fluid, synovium, and surface layers of the cartilage.[6] In previous studies we have found that lining macrophages are of utmost importance in both onset and prolongation of experimental murine arthritis. When synovial intima macrophages were selectively depleted from the knee joint either before induction or during immune complex (IC)-mediated arthritides like collagen type II or antigen-induced arthritis (AIA), onset and course of arthritis was largely reduced.[7–9]

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IgG-containing ICs communicate with lining macrophages using FcγR.[10] In the mouse, three classes of FcγR have been described. FcγRI and III are activating receptors and lead to elevation of intracellular signaling after binding of ICs.[11–12] The third class is FcγRII, which can co-ligate with either FcγRI or FcγRIII, resulting in inhibition of intracellular signaling.[13] Coordinate expression of activating and inhibiting FcγR on synovial lining cells has been shown to regulate both joint inflammation and severe cartilage destruction.[10] The inhibiting FcγRII exists as two isoforms, FcγRIIb1 and FcγRIIb2, differing by a 47-amino acid insertion in the intracytoplasmatic domain of FcγRII encoded by the first exon of the FcγRII gene.[14] The *in vivo* role of FcγRII was extensively studied using FcγRII-deficient mice and it is generally agreed that inhibition occurs only when FcγRII is co-clustered with ITAM-bearing receptors.[15–16] The inhibitory function is mediated by the inositol phosphatase SHIP which associates with the phosphorylated ITIM of FcγRII via the SHIP SH2 domain[17]; however, *in vitro* studies also suggested other biological functions for FcγRII. By transferring cDNA of both FcγRII isoforms into fibroblastic cell lines which do not express FcγR, it was found that FcγRIIb2 is involved in endocytosis and enhancement of antigen presentation.[18–20] FcγRIIb1, which is preferentially expressed in B lymphocytes, lacks immune internalization properties, yet it inhibits B-cell activation and subsequent antibody production when cross-linked to membrane Ig. This suggests that FcγRII, apart from inhibiting activating FcγR, may also have other important functions *in vivo*.

In the present study, we investigated the *in vivo* role of FcγRII, uncoupled from its function as inhibitor of activating FcγR, in regulating joint inflammation and severe cartilage destruction in models of IC-mediated arthritis using mice which were made deficient for either both activating FcγR (FcγRI/III−/−) or all three FcγR (FcγRI/II/III−/−). We found that FcγRII is a major regulator of joint inflammation by promoting clearance of ICs by synovial lining cells. Furthermore activating FcγR on inflammatory cells appeared to be prerequisites for severe irreversible cartilage destruction.

Materials and Methods

Animals

FcγRI and FcγRIII−/− were made deficient for the ligand-binding α-chain of FcγRI[21] and FcγRIII,[22] respectively. FcγRIII−/− were back-crossed to the C57BL/6 background for 12 generations. FcγRIIb−/− were developed by Dr. Takai[15] in the 129/Ola (H-2b) and C57BL/6 (H-2b) background. FcγRI−/− was made in the 129Ola/C57BL/6 background. FcγRII/III−/− and their controls (control 1) were developed in the 129Ola/C57BL/6 background. Intercrossing led to FcγRI/II/III −/− in the 129Ola/C57BL/6/balb/c (enriched for C57BL/6), as were their triple controls (control 2). Control C57BL/6 and 129Ola/C57BL/6 were derived from Jackson laboratories (Bar Harbor, ME) and bred in our own facilities. Homozygous mutants and their wild-type (WT) controls, aged 10 to 12 weeks, were used in the experiments.

Humoral Immunity Against mBSA

Antibodies of various isotypes (IgG, IgG1, IgG2a, IgG2b, IgG3) directed against methylated bovine serum albumin (mBSA) were measured in sera of individual mice with an enzyme-linked immunosorbent assay (ELISA). Antigen was coated on microtiter plates (Greiner, Alphen a/d Rijn, The Netherlands) at a concentration of 100 μg/ml. Antibody titers were assessed by two-fold serial dilution of the sera followed by detection of bound mouse Ig with 1:500 diluted peroxidase-conjugated rabbit anti-mouse Ig (Miles Laboratories Inc., Elkhart, IN). O-Phenylenediamine (1 mg/ml; Sigma, St. Louis, MO) was used as substrate for peroxidase, and the antibody titer was determined by using 50% of the maximal extinction as an end-point.

Cellular Immunity Against mBSA

Mouse spleen cells were isolated and washed in RPMI supplemented with 10% fetal calf serum, glutamin (2 mmol/L), and pyruvate (1 mmol/L). Erythrocytes were lysed by treatment of the cells with an 0.16 mol/L NH_4CL solution in 0.17 mol/L Tris, pH 7.2, for 5 minutes. After two washes in RPMI, the cells were plated on plastic T flasks (75 mm^2) from Falcon Plastics (Oxnard, CA). After 60 minutes of incubation at 37°C, the nonadherent cells were harvested by aspiration and two 4- to 5-ml RPMI washes of the adherent cells. 100 μl of RPMI containing 1×10^5 T-cell-enriched spleen cells were placed in each well of a sterile, U-bottomed polystyrene microculture plate (Costar, Cambridge, MA). Antigens or mitogens were added in another 100 μl to give a total volume of 200 μl, and final concentrations of antigen of 50, 25, 12, 6, and 3 μg/ml. Cultures were maintained at 37°C in a humified atmosphere of 2% CO_2 and 98% air for 4 days. Sixteen hours before harvesting, 1 μCi of [^{3}H]-thymidine (6.7 Ci/mmol from New England Nuclear, Boston, MA) was added in 25 μl of RPMI. Cultures were harvested with a cell harvester (Tomtec, Hamden, CT) and [^{3}H]-thymidine incorporation was determined.

Induction of Experimental Arthritis

AIA was induced by injecting 60 μg of mBSA in 6 μl PBS directly into the knee joints of mice that were previously immunized with that antigen. Mice were immunized with 100 μg of mBSA (Sigma), emulsified in 100 μl Freund's complete adjuvant. Injections were divided over both flanks and footpath of the forelegs. Heat-killed *Bordetella pertussis* was administered intraperitoneally as an additional adjuvant. Two subcutaneous booster injections with 50 μg mBSA/CFA were given in the neck region 1 week after the initial immunization. Two weeks after these injections, arthritis was induced into the right knee joint, resulting in chronic arthritis.

Immune complex arthritis (ICA) was passively induced in knee joints of mice.[23] Three micrograms of lysozyme in 6 μl were injected directly into the knee joint of mice that previously were given anti-lysozyme antibodies intravenously. An acute arthritis develops, which became maximal at day 3 and waned thereafter.

A non-IC-mediated zymosan-induced arthritis (ZIA) was induced by injecting 180 μg of sterilized zymosan in 6 μl PBS into the knee joint.

^{99m}Tc Uptake Measurements

Joint inflammation was measured by ^{99m}Tc pertechnetate uptake in the knee joint. This method was shown earlier to correlate well with histological findings.[24] Briefly, mice were injected intraperitoneally with 12 μCi ^{99m}Tc and subsequently sedated with chloralhydrate. Thirty minutes thereafter, gamma radiation was assessed by use of a collimated Na-I-scintillation crystal with the knee in a fixed position. Arthritis was scored as the ratio of the ^{99m}Tc uptake in the right (R) and the left (L) knee joint. R:L ratios >1.1 were taken to indicate inflammation of the right knee joint.

Histology

Total knee joints were dissected, fixed in phosphate-buffered formalin (pH 7.4), decalcified in 5% buffered formic acid, and subsequently embedded in paraffin wax. Semiserial frontal whole knee joint sections (7 μm) were stained with hematoxylin and eosin (H&E) or safranin-O and Fast Green. The severity of joint inflammation was determined using an arbitrary score (0 to 3). Infiltrate and exudate were scored separately. Scoring was performed in a blinded manner by two independent observers: 0, no cells; 1, mild cellularity; 2, moderate cellularity; 3, maximal cellularity.

Endocytosis and Clearance of IgG Immune Complexes

Endocytosis and clearance of IgG ICs were studied both *in vivo* and *in vitro*. The clearance of IgG-containing ICs from arthritic knee joints of FcγRI/II/III−/− and FcγRI/III−/− mice was studied using anti-IgG immunolocalization. In one group, AIA was induced whereas in a second group knee joints were injected with 6 μg of heat-aggregated IgG. The latter was made by heating rabbit-IgG during 30 minutes at 61°C. Knee joints were isolated 7 days after AIA induction or 8 hours after injection of aggregated IgG. Paraffin-embedded total knee joint sections were pretreated with hyaluronidase ABC and additionally stained with either goat anti-mouse peroxidase or goat anti-rabbit peroxidase overnight. Development of the peroxidase product was done using diaminobenzidine (0.5 mg/ml). Sections were counterstained with H&E.

Isolation of Peritoneal Macrophages from Mice Previously Injected with Thioglycolate

ICs were preformed by incubating soluble fluorescein isothiocyanate (FITC)-labeled OVA (Molecular Probes, Leiden, The Netherlands) with 25 μg/ml polyclonal OVA-specific rabbit IgG (rIgG OVA; Sigma-Aldrich, Zwÿndrecht, The Netherlands) for 30 minutes at 37°C in polypropylene tubes. Fifty thousand peritoneal macrophages were added to FACS tubes containing OVA-ICs or soluble OVA and incubated for 15 minutes at 37°C. Cells were washed twice and resuspended in presence of 0.4% (w/v) trypan blue (Sigma-Aldrich), which quenches extracellular, but not intracellular, fluorescence. Flow cytometry was performed with FACScan. The mean fluorescence value of six measurements is shown.

Immunohistochemical Staining of Myeloid-Related Proteins MRP8 and 14

Rabbit anti-sera against recombinant murine MRP8 (α-MRP8) and MRP14 (α-MRP14) were produced as described earlier.[25] Monospecificity of antibodies was analyzed by immunoreactivity against recombinant MRP8 and MRP14 and Western blot analysis of lysates of granulocytes.[25] Formalin-fixed sections of knee joints were stained using a final antibody concentration of 1 μg/ml. Primary antibodies were detected using peroxidase-conjugated second-stage antibodies against rabbit IgG (Dianova). Finally, sections were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany). MRP8 and MRP14-positive cells present in the joint cavity and synovial lining were determined as expressed as percentage of the total cell population, using an arbitrary score (0:0%, 1:1 to 30%, 2:31 to 70%, 3:71 to 100%).

Immunolocalization of MMP-Induced Neoepitope (VDIPEN)

For immunohistochemical analysis, sections were deparaffinized, rehydrated, and digested with chondroitinase ABC (Sigma; 0.25 U/ml, 0.1 mol/L Tris-HCL, pH 8.0) for 1 hour at 37°C, to remove chondroitine sulfate from the proteoglycans. Sections were then treated with 1% H_2O_2 in methanol for 20 minutes and subsequently for 5 minutes with 0.1% (v/v) Triton X-100 in PBS. After incubation with 1.5% (v/v) normal goat serum for 20 minutes, sections were incubated with affinity-purified anti-VDIPEN IgG overnight at 4°C. These antibodies were kindly given by Irwin Singer and Ellen Bayne (Merck Research Laboratories, Rahway, NJ) and have been extensively characterized before.[26,27] In addition, sections were incubated with biotinylated goat anti-rabbit IgG and binding-detected using avidin-peroxidase staining (Elite kit, Vector Laboratories, Inc., Burlingame, CA). Development of the peroxidase product was done using nickel enhancement and counterstaining was done with orange G (2%) for 5 minutes.

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Table 1. Cellular and Humoral Immunity Four Weeks after Immunization with mBSA/CEA

	Cellular immunity (SI)					Humoral immunity (titer)				
	50	25	12	6	3	IgG	IgG1	IgG2a	IgG2b	IgG3
WT	3 (1)	5 (1)	6 (2)	7 (1)	5 (1)	13 (2)	11 (1)	12 (2)	11 (1)	10 (1)
FcγRI/III−/−	5 (2)	7 (1)	8 (1)	8 (0)	5 (0)	14 (2)	13 (1)	13 (1)	11 (1)	11 (0)
WT	1 (1)	2 (1)	5 (0)	6 (1)	4 (1)	17 (1)	14 (2)	13 (1)	14 (0)	10 (1)
FcγRI/II/III−/−	1 (0)	4 (2)	6 (1)	7 (1)	5 (0)	18 (1)	16 (2)	15 (1)	14 (1)	11 (2)

Cellular immunity was measured by spleen lymphocyte proliferation and expressed as stimulation index (ratio of T cells stimulated with various concentrations of mBSA (50, 25, 12, 6, 3 μg/ml) and T cells not stimulated. Values represent the mean ± SD of three groups of two mice each. Humoral immunity was measured as antibody production against mBSA and various isotypes were determined using ELISA. The sera of six mice were individually tested. Values represent the mean titer ± SD. Note that IgG1, IgG2a, and IgG3 levels were elevated (2–4 times) whereas IgG2b was not elevated in both FcγRI/III−/− and FcγRI/II/III−/− when compared to controls.

Measurement and Characterization of Chondrocyte Death

Chondrocyte death was determined at day 7 after AIA induction in total knee joint sections stained with H&E. Chondrocyte death was determined as percentage of the area of the cartilage containing empty lacunae in relation to the total area.

Results

Role of the Inhibitory FcγRII in the Absence of Activating FcγR During Antigen-Induced Arthritis

To investigate the role of FcγRII in the absence of activating FcγR, we induced AIA in knee joints of FcγRI/III−/− and FcγRI/II/III−/− mice. As the absence of FcγR may alter the immunological response against methylated BSA during immunization of these mice, thereby impairing the onset and course of arthritis, we first tested cellular and humoral immunity to mBSA, 3 weeks after immunization. Cellular immunity, as measured by spleen lymphocyte stimulation (LST) against various concentrations of mBSA, showed no significant differences between knockout (KO) and their controls (Table 1). In addition, humoral immunity was measured by ELISA. Total IgG, IgG1, IgG2a, and IgG3 anti-mBSA levels were two to four times higher, whereas IgG2b levels were not different in sera of both immunized FcγRI/III−/− and FcγRI/II/III−/− when compared to their WT controls (Table 1).

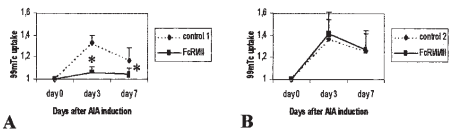

Figure 1. Inflammation of the knee joint, expressing cellular activation, was determined as R:L ratios of ^{99m}Tc uptake at various days (3 and 7) after injection of 60 μg mBSA in the right knee joints of mBSA-immunized FcγRI/III−/− (**A**) and FcγRI/II/III−/− mice (**B**) and were compared to control 1 and control 2, respectively. In the left knee joints PBS was injected. Values represent the mean ± SD of seven mice. Data were evaluated using the Wilcoxon rank test (*, $P \leq 0.05$). Note that swelling was almost absent in knee joints of arthritic FcγRI/III−/−, whereas similar joint inflammation was found in arthritic FcγRI/II/III−/−.

Subsequently, AIA was induced and knee joint swelling was determined at various time-points after induction. We found that inflammation in arthritic knee joints of FcγRI/III−/− was significantly lower both at day 3 and day 7 when compared to arthritic controls (Figure 1A). Interestingly, inflammation in arthritic knee joints of FcγRI/II/III−/− was not different from that seen in control knee joints both at day 3 and 7 after AIA induction (Figure 1B).

To further verify these observations, histology of total arthritic knee joints was investigated. At day 7, FcγRI/III−/− mice showed that although IgG2a antibody titers were much higher, exudate and infiltrate was significantly lower (90% and 87%, respectively) when compared to WT controls (Figure 2, A and D, *versus* WT control, Figure 2E). In contrast, at day 7 after AIA induction in knees of FcγRI/II/III−/−, joint inflammation appeared to be markedly higher when compared to their controls (exudate and infiltrate were respectively 200% and 120% higher (Figure 2, B and F, *versus* WT control, Figure 2E) suggesting that FcγRII is an important regulator of joint inflammation in the absence of activating FcγR.

FcγRII Regulation of Joint Inflammation Is Specific for Immune Complexes

To further investigate whether FcγRII regulation of joint inflammation in the absence of activating FcγR is specific for ICs (and not, for example, by T cells also involved in AIA), we induced arthritis solely by ICs. ICA was passively induced by injecting lysozyme in knee joints of mice that were previously given anti-lysozyme antibodies. Histology taken at day 3 after arthritis induction showed that joint inflammation was almost completely prevented in FcγRI/III−/−, whereas substantial arthritis was found in their WT controls (Figure 3A). When ICA was induced in knee joints of FcγRI/II/III−/−, the inflammatory cell mass as measured at day 1 and 3 was in line with that found in AIA, again significantly higher when compared to WT controls. At day 1, exudate and infiltrate were 310% and 60%, respectively (Figure 3B), and at day 3, 2200% and 270% higher (Figure 3C).

To further substantiate the specificity for ICs, we additionally injected zymosan in the knee joints of FcγRI/II/III−/−. The inflammatory cell mass measured at day 3 after ZIA induction was not different from controls, suggesting that knee joints of these mice develop a normal

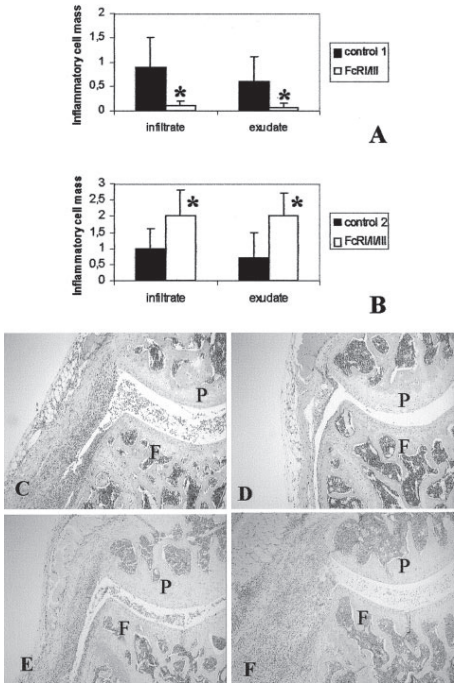

Figure 2. Frontal sections of whole knee joints 7 days after induction of AIA in FcγRI/III−/− (**A**), FcγRI/II/III−/− (**B**), and their WT controls 1 and 2. The amount of cells present in the synovium (infiltrate) and in the knee joint cavity (exudate) was determined using an arbitrary scale from 0 to 3: 0, no cells; 1, minor; 2, moderate; 3, maximal. The amount of cells was determined independently by two blinded observers. Data are the mean of seven mice. Significance was tested using the Wilcoxon rank test (*, $P < 0.05$). Original magnification of the photographs is ×100. F, femur; P, patella. Note the significantly lower inflammatory cell mass in knee joints of FcγRI/III−/− (**A** and **D** *versus* WT control **C**) and the significantly higher cell mass in FcγRI/II/III−/− knee joints (**B** and **F** *versus* WT control **E**) when compared to WT controls.

inflammatory response after injection with zymosan (Figure 3D).

FcγRII Is Involved in Efficient Endocytosis and Clearance of ICs

One of the reasons why joint inflammation is elevated in FcγRI/II/III −/− may be an impaired endocytosis and clearance of ICs from the joint.

To investigate whether FcγRII is involved in endocytosis of IgG containing ICs, we first investigated the presence of murine IgG, localized within the arthritic joints at day 7 after AIA induction using immunolocalization. No significant differences in IgG deposition was found in the knee joints of FcγRI/III−/− and their WT controls, suggesting an effective clearance of IgG containing ICs in the presence of only FcγRII (Figure 4B *versus* WT controls, Figure 4A). In contrast, arthritic knee joints of FcγRI/

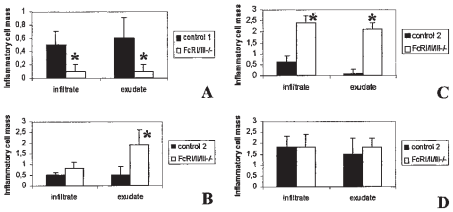

Figure 3. Frontal sections of whole knee joints, 3 days after induction of ICA in FcγRI/III−/− (**A**), 1 and 3 days after ICA in FcγRI/II/III−/− (**B** and **C**) or 3 days after induction of zymosan-induced arthritis (**D**) in FcγRI/II/III−/− and their WT controls 1 and 2. The amount of cells present in the synovium (infiltrate) and in the knee-joint cavity (exudate) was determined using an arbitrary scale from 0 to 3: 0, no cells; 1, minor; 2, moderate; 3, maximal. The amount of cells was determined independently by two blinded observers. Data are the mean ± SD of seven mice. Significance was tested using the Wilcoxon rank test (*, $P < 0.05$). Original magnification, ×100. F, femur; P, patella. Note the significantly lower inflammatory cell mass after ICA induction in knee joints of FcγRI/III−/− (**A**) and the significantly higher cell mass at day 1 and 3 in FcγRI/II/III−/− knee joints (**B** and **C**) and the comparable cell mass at day 3 after ZIA (**D**) when compared to WT controls.

II/III−/− contained large amounts of IgG, suggesting that removal of ICs is retarded (Figure 4D *versus* WT controls, Figure 4C). To confirm this finding, heat-aggregated IgG was injected in the knee joints of FcγRI/III−/− and FcγRI/II/III−/− mice and their WT controls. Immunolocalization of IgG showed that 8 hours after injection significantly more IgG was detected in FcγRI/II/III−/− knee joints which was mainly bound to the synovial lining layer (Figure 5D *versus* WT control, Figure 5E). Similar intensity of staining was found when aggregated IgG was injected in knee joints of FcγRI/III−/− and their controls (Figure 5B *versus* WT control, Figure 5A).

To further analyze FcγRII function on macrophages, thioglycollate-induced peritoneal macrophages were isolated. When macrophages expressing only FcγRII (FcγRI/III−/−) were pre-incubated with pre-formed FITC-

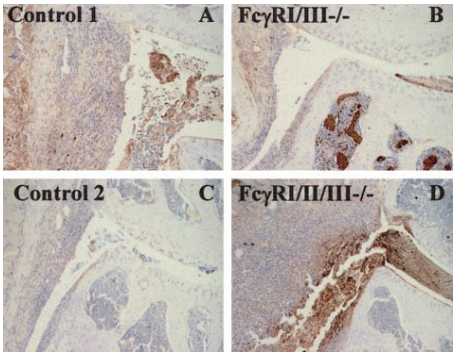

Figure 4. Presence of IgG containing ICs, detected by immunolocalization, in knee joints of various KO mice, 7 days after induction of antigen-induced arthritis. Note that IgG is present in large amounts in arthritic FcγRI/II/III−/− knee joints when compared to their WT controls 2 (**D** *versus* WT control **C**). No difference in amounts of IgG was found in arthritic knee joints of FcγRI/III−/− when compared to their controls 1 (**B** *versus* WT control **A**). Original magnification, ×100.

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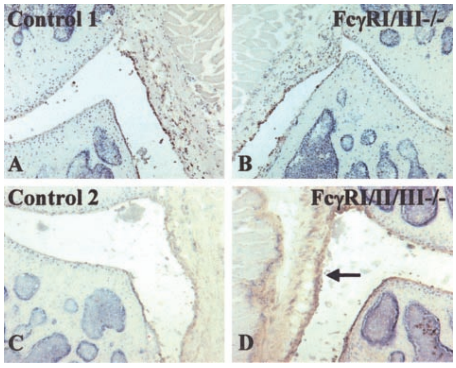

Figure 5. Presence of heat-aggregated IgG detected by immunolocalization, 8 hours after injection in knee joints of various KO mice. Heat-aggregated rabbit IgG is less efficiently cleared (**arrow**) when injected in FcγRI/II/III−/− knee joints in comparison to WT controls 2 (**D** *versus* WT control **C**). No differences are found between knee joints of FcγRI/III−/− and their WT controls 1 (**B** *versus* WT control **A**). Original magnification, ×100.

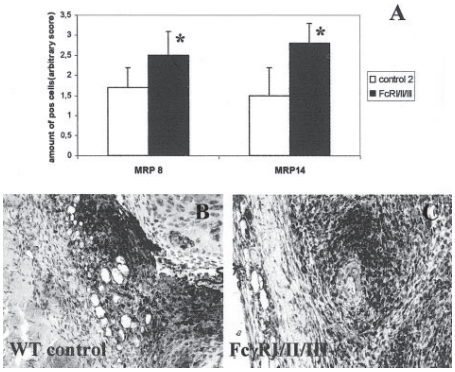

Figure 7. Expression of activation markers MRP8 and 14 in synovial lining and joint cavity in FcγRI/II/III−/− and their WT controls 2 at day 7 after AIA induction. Note the significantly higher expression of both MRP8 (**A** and **C** *versus* WT control **B**) and MRP14 (**A**). Data are the mean of seven mice. Significance was tested using the Wilcoxon rank test (*, $P < 0.05$). Original magnification, ×400.

labeled OVA-IgG ICs, prominent endocytosis was found not different from control macrophages (Figure 6B *versus* control, Figure 6A). Interestingly when FcγRII was also absent (FcγRI/II/III−/−), endocytosis of ICs was completely prevented (Figure 6C *versus* control, Figure 6A).

Type and Activation State of Inflammatory Cells in Arthritic Knee Joints of FcγRI/II/III−/−

To further analyze the composition of the inflammatory cell mass within the arthritic FcγRI/II/III−/− knee joint, we next investigated the type and activation state of the inflammatory cells using immunolocalization. PMN and monocyte/macrophage ratios were determined by immunolocalization using NIMP-R14, which stains PMN specifically. At day 7 after AIA induction in FcγRI/II/III−/− knee joints, the majority of inflammatory cells appeared to be monocytes (ratio monocytes/PMN 60–40) and no differences were found between KO and their controls. In addition, we determined the pro-inflammatory phenotype of the infiltrated cells. In FcγRI/II/III−/− mice, the infiltrated cells in the arthritic joint displayed an activated phenotype, according to high expression of MRP8 and 14 (respectively 47% and 92%: Figure 7, A and C *versus* WT control, Figure 7B), whereas infiltrated cells in the joints of FcγRI/III−/− mice failed to express these activation markers (data not shown). In fact this implies that FcγRII can prevent cellular activation even in the absence of FcγRI/III.

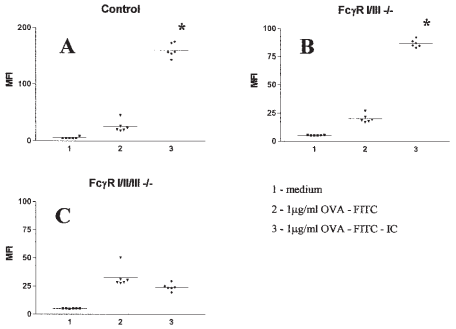

Figure 6. Endocytosis of soluble fluorescent ovalbumin-IgG ICs by peritoneal macrophages derived from FcγRI/III−/− and FcγRI/II/III−/−. Note that IC are efficiently endocytosed by macrophages expressing only FcγRII (FcγRI/III−/−) not different from controls (**B** *versus* WT control **A**). When macrophages missing all FcγR (FcγRI/II/III−/−) were used, endocytosis of ICs was blocked (**C** *versus* WT control **A**). Peritoneal macrophages of WT littermates of FcγRI/III−/− and FcγRI/II/III−/− were used as controls. As uptake of IC by both control cells was similar, only control cells of FcγRI/II/III−/− are shown. MFI, mean fluorescent intensity. Data are the mean of three different experiments. Significance was tested using the Wilcoxon rank test (*, $P < 0.05$).

Activated Inflammatory Cells in the Absence of Activation FcγR Fail to Induce Severe Cartilage Destruction

As the majority of the infiltrated cells in the FcγRI/II/III −/− knee joints were activated we additionally investigated whether these cells were capable of inducing severe cartilage destruction like metalloproteinase (MMP)-induced damage and chondrocyte death. MMPs are crucial in degradation of aggrecan and collagen, leading to irreversible cartilage destruction. MMPs degrade aggrecan leaving the C-terminal ending with the amino acid sequence VDIPEN which can be detected by specific antibodies around day 5 after induction of AIA.[27] For this reason, AIA day 7 was taken to detect VDIPEN expression in the cartilage matrix.

The amount of VDIPEN was measured by determining the percentage of the area of cartilage expressing VDIPEN. In most investigated knee joints of WT mice

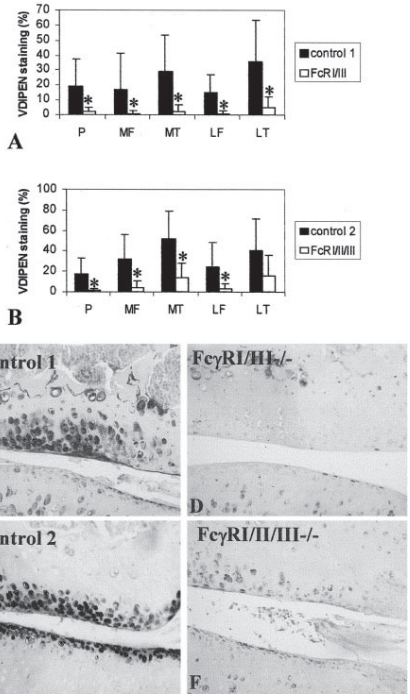

Figure 8. Expression of VDIPEN neoepitopes in knee joints of FcγRI/III−/− (**A**), FcγRI/II/III−/− (**B**) and their WT controls 1 and 2, 7 days after AIA induction. VDIPEN staining was determined in various cartilage layers of the knee joint (P, patella; MF, medial femur; MT, medial tibia; LF, lateral femur; LT, Lateral tibia). VDIPEN was expressed as percent positive staining of the total cartilage area. VDIPEN staining was almost absent in arthritic knee joints of both FcγRI/III−/− (**A** and **D** *versus* WT control **C**) as in FcγRI/II/III−/− (**B** and **F** *versus* WT control **E**). Data represent the mean ± SD of seven mice and were statistically evaluated using the Wilcoxon rank test. $^{*}P < 0.05$.

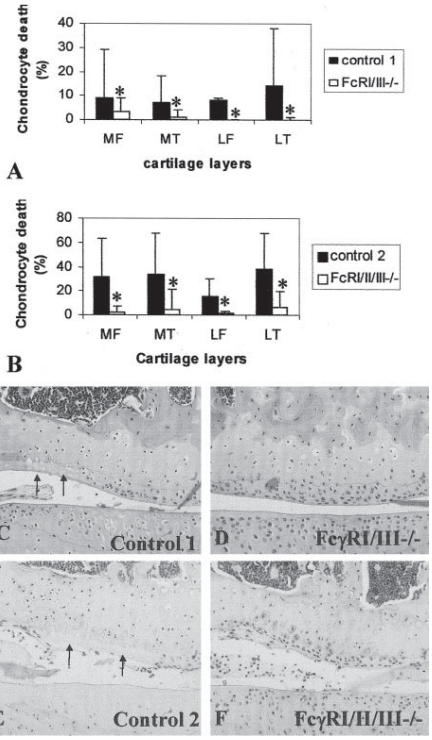

Figure 9. Measurement of chondrocyte death in cartilage layers of knee joints of FcγRI/III−/− (**A**), FcγRI/II/III−/− (**B**) and their WT controls 1 and 2, 7 days after AIA induction. Chondrocyte death was determined in various cartilage layers of the knee joint (MF, medial femur; MT, medial tibia; LF, lateral femur; LT, lateral tibia). Chondrocyte was expressed as percent of empty lacunae (**arrows** in **C** and **E**) of the total cartilage area. Note that chondrocyte death was significantly lower in both FcγRI/III−/− (**A** and **D** *versus* WT control **C**) as in FcγRI/II/III−/− (**B** and **F** *versus* WT control **E**). Data represent the mean ± SD of seven mice and were statistically evaluated using the Mann-Whitney *U*-test. *, $P < 0.05$.

injected with 60 μg of mBSA, VDIPEN staining was found particularly in the cartilage layers of tibia and femur (Figure 8,A, C, and E). In arthritic knees of FcγRI/III−/−, VDIPEN expression was completely absent when comparable to WT controls (Figure 8, A and D, *versus* WT control, Figure 8C). Interestingly, in FcγRI/II/III−/− arthritic joints, although much more joint inflammation was found which abundantly expressed the activation markers MRP8 and 14, VDIPEN was virtually absent when compared to arthritic controls (Figure 8, B and F, *versus* WT control, Figure 8E). This confirms again that only activating FcγR mediates severe cartilage destruction, and that this prerequisite also holds in condition of abundant inflammatory cell influx showing an activated phenotype.

In addition, chondrocyte death was measured by determining the percentage of the area of cartilage with empty lacunae. Chondrocyte death varied between 5% to 40% in various cartilage layers of the WT arthritic knee joints (Figure 9, A, C, and E). Chondrocyte death was absent in arthritic FcγRI/III−/− knee joints (Figure 9, A and D, *versus* WT control, Figure 9C). Again in arthritic FcγRI/II/III−/−, despite its high joint inflammation, chondrocyte death was completely absent (Figure 9, B and F, *versus* WT control, Figure 9E).

Discussion

The *in vivo* role of FcγRII has been extensively studied using FcγRII KO mice. Induction of IC-mediated inflammation within these mice caused a significantly elevated inflammation when compared to controls. In general, the function of FcγRII as a major inhibitor of the activatory FcγR is highlighted. In the present study we demonstrate that in the absence of activating FcγR, the inhibiting FcγRII still functions as an important down-regulator of

synovial inflammation which might be related to IC clearance and complement activation.

FcγRII, in the absence of activating FcγR significantly reduced joint inflammation during T cell-mediated AIA. One of the explanations may be an altered T cell response. FcγR are expressed on precursors of T cells[28] and the absence of these receptors may have had an impact on the development of T cell reactivity and may explain the markedly elevated anti-mBSA IgG2a antibody responses in both immunized FcγRI/III−/− as FcγRI/II/III−/−. However anti-mBSA T cell responses were found not to be significantly different and this may indicate that FcγRII present on resident synovial lining macrophages may be more important in regulating joint inflammation.

To further substantiate the involvement of FcγRII on synovial macrophages, arthritis was induced by local deposition of IC within the joint. In that model, arthritis is regulated by lining macrophages and not by T cells.[23] Comparable to that seen in AIA, FcγRII, in the absence of activating FcγR, again strongly reduced joint inflammation. The FcγRII dependency appeared to be IC-specific since injection of zymosan directly into the knee joint of FcγRI/II/III−/− caused similar joint inflammation than when injected in WT and thus indicates that the joints of these mice develop a normal inflammatory response on non-IC triggers.

The most plausible function of FcγRII in joint inflammation in the absence of activating FcγR is its role in clearance of IgG ICs from the joint. Clearance of IC is largely regulated by synovial lining cells and its efficiency is highly correlated to development of arthritis.[29] IgG-containing ICs activate complement. In the mouse IgG2a and IgG3 mediate complement via the classical pathway,[30] whereas IgG1, when attached to cartilage layers uses the alternative pathway.[31] Co-dominance between complement and FcγR has previously been described.[32] Efficient removal of these ICs from the joint may lower the amount and course of complement activation within the joint, thereby lowering onset and/or prolongation of arthritis. This is in line with studies that show that complement is especially important in the onset, whereas at later time-points inflammation is more FcγR-mediated.[33]

Within the joint, macrophages are crucial in clearance and endocytosis of ICs. In earlier *in vitro* studies using cDNA transfection, it was found that FcγRII mediates internalization and lysosomal degradation of IgG-antigen complexes.[34–35] In line with that, we now find that peritoneal macrophages from KO mice, which only express FcγRII and no activating FcR, are still able to endocytose soluble ovalbumin-IgG complexes not different from controls, whereas in the absence of all FcγR, endocytosis is almost completely blocked. Moreover when ICs were injected directly into the joint, clearance and endocytosis by lining cells were strongly retarded in the FcγRI/II/III−/−. This suggests that *in vivo* FcγRII is a major receptor for endocytosis. *In vivo* studies using FcγRII-deficient mice have shown that FcγRII inhibited phagocytosis and clearance, and this was explained by inhibiting activating FcγR.[36] We now clearly demonstrate for the first time that *in vivo*, FcγRII also reduces inflammation by accelerating IC clearance and endocytosis. In contrast to our study, Mathis et al[31] found no involvement of Fcγ II in the K/BXN serum transfer arthritis model. One explanation may be that arthritis within this model is regulated by anti-GPI antibodies of only the IgG1 isotype. These antibodies preferentially bind to FcγRIII which may largely be responsible for IC removal within this model.

Removal of IC from the joint is a combined action of leakage through the pores of the lining layer into the draining lymph vessels and lymph nodes, and binding and endocytosis by synovial lining cells. Synovial intima macrophages first meet these ICs and have been shown to be crucial in both onset as well as propagation of synovial inflammation.[8,37] This in contrast to synovial intima fibroblasts which fail to express FcγR. Activation of lining cells forms one of the crucial events in arthritis development. Transfer of early activated lining cells appeared to be sufficient to induce arthritis in normal rats.[38]

It is generally accepted that FcγRII acts by coligating with activatory FcγRIII and probably also with FcγRI eventually leading to inactivation of synovial macrophages and reduced production of cytokines and chemokines.[16]In the present study we find that in the absence of all FcγR, IgG-ICs when injected into the joint still bind to intimal synovial cells. Moreover these cells express abundant MRP8/14 indicating that they still become activated and likely produce sufficient pro-inflammatory factors leading to pronounced joint inflammation. As FcγR are absent, IC may bind to other receptors. A good candidate may be the promiscuous complement receptor 3 (CR3).[39] The complement splitting product C3bi tightly binds to various antibody isotypes involved in IC formation.[40] C3bi may form the link between IC and binding to the CR3 receptor on macrophages not expressing FcγR and may mediate intracellular signaling leading to activation of the intimal macrophage.

The amount of inflammatory cell mass within an inflamed joint is often related to severe cartilage destruction. In the present study we found a remarkable uncoupling between joint inflammation and severe cartilage destruction like MMP-mediated damage and chondrocyte death. Severe cartilage destruction seen during IC-mediated arthritides is mediated by metalloproteinases, which are released by chondrocytes in a latent pro-form within the cartilage matrix. Interleukin-1 appeared to be the master cytokine regulating MMP production by the chondrocyte.[41] Large amounts of inactive MMPs accumulate within the cartilage matrix and on activation lead to destruction of the collagen type II network and the proteoglycans embedded within this matrix.[42] The factors needed for this activation step are still unknown.

As inflammatory cells are capable of mediating activation of latent MMPs inside the cartilage matrix,[43] the way in which these cells become activated within the joint seems crucial and recent studies by our lab suggest that activating FcγR are of utmost importance.[10,21,44]

Binding of IC to activating FcγR (especially FcγRI) on macrophages may lead either to a higher production of MMP-activating factors or promote generation of mediators which inhibit these factors. MMP-activating factors may be other MMPs,[45] enzymes like plasmin,[46] EMPRINN,[47] or oxygen radicals,[48] which all have been

shown to possess the capacity of activating latent MMPs. Oxygen radicals have been shown to be abundantly released by macrophages after IC binding to FcγRI[49] and may explain the clear chondrocyte death seen during IC-mediated arthritis. In the absence of FcγRI, chondrocyte death was completely absent[10,44] at day 7 after AIA induction. In contrast, FcγR binding may also lead to a rise in inhibitors like TIMPs, which may efficiently reduce MMP-mediated cartilage destruction.

When infiltrating cells are activated by bacterial or yeast cell walls when injected into the knee joint of mice, although a pronounced inflammation developed, MMP-mediated cartilage destruction nor chondrocyte death was detected.[27] Although these cells express an inflammatory phenotype, the released factors were incapable of activating latent MMP, which were found in large amounts within the cartilage layers of the joint.[27] In line with this we now find that in knee joints of arthritic triple KO, despite abundant joint inflammation, no VDIPEN epitopes nor chondrocyte death was observed. Like in the non-IC arthritis, the infiltrated cells despite expressing an inflammatory phenotype were incapable to activate MMPs. This again confirms that FcγR activation is a prerequisite for inducing irreversible cartilage destruction.

The present study underlines that activating FcγR are crucial in induction of severe cartilage destruction and that FcγRII is an important inhibiting receptor which regulates both chronic joint inflammation as well as cartilage destruction during arthritis. FcγRII may be a powerful inhibitor to prevent both synovial inflammation and cartilage destruction and its overexpression may form a new therapeutic tool to combat the severe pathogenicity of ICs involved in RA.

References

1. Primer on the Rheumatic Diseases. Edited by JH Klippel. Atlanta, Georgia, Arthritis Foundation, 1997
2. Mulherin D, Fitzgerald O, Bresnihan B: Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996, 39:115–124
3. Zvaifler NJ: Macrophages and the synovial lining. Scand J Rheumatol Suppl 1995, 101:67–75
4. Brennan FM, Maini RN, Feldmann M: Role of pro-inflammatory cytokines in rheumatoid arthritis. Springer Semin Immunopathol 1998, 20:133–147
5. Szekanecz Z, Strieter RM, Kunkel SL, Koch AE: Chemokines in rheumatoid arthritis. Springer Semin Immunopathol 1998, 20:115–132
6. Cooke TDV: Mechanism of cartilage destruction: relation to choice of therapeutic agent. Semin Arthritis Rheum 1985, 51(Suppl 1):16–23
7. van Lent PLEM, Holthuysen AEM, van den Bersselaar LAM, van Rooijen N, Joosten LAB, van de Loo FAJ, van de Putte LBA, van den Berg WB: Phagocytic lining cells determine local expression of inflammation in type II collagen-induced arthritis. Arthritis Rheum 1996, 39:1545–1555
8. van Lent PLEM, van den Hoek AEM, van den Bersselaar LAM, Spanjaards MFR, van Rooijen N, Dijkstra CD, van de Putte LBA, van den Berg WB: In vivo role of phagocytic synovial lining cells in onset of experimental arthritis. Am J Pathol 1993, 143:1226–1237
9. van Lent PLEM, Holthuysen AEM, van Rooijen N, van de Loo FAJ, van de Putte LBA, van den Berg WB: Phagocytic synovial lining cells regulate acute and chronic joint inflammation after antigenic exacerbation of smouldering experimental murine arthritis. J Rheumatol 1998, 25:1135–1145
10. Nabbe KCAM, Blom AB, Holthuysen AEM, Boross P, Roth J, Verbeek S, van Lent PLEM, van den Berg WB: Coordinate expression of activating Fcγ receptors I and III and inhibiting Fcγ receptor IIB in the determination of joint inflammation and cartilage destruction during immune complex-mediated arthritis. Arthritis Rheum 2003, 48:255–265
11. Ravetch JV, Kinet JP: Fc receptors. Annu Rev Immunol 1991, 9:457–492
12. Ravetch JV: Fc receptors: rubor redux. Cell 1994, 78:553–560
13. Fridman WH, Bonnerot C, Daeron M, Amigorena S, Teillaud JL, Sautes C: Structural bases of Fcγ receptor functions. Immunol Rev 1992, 125:49–76
14. Chen L, Pielak GJ, Thompson NL: The cytoplasmic region of mouse FcγRIIb1, but not FcγRIIb2, binds phospholipid membranes. Biochem 1999, 38:2102–2109
15. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV: Augmented humoral and anaphylactic responses in Fcγ deficient mice. Nature 1996, 379:346–349
16. Yuasa T, Kubo S, Yoshino T, Ujike A, Matsumura K, Ono M, Ravetch JV, Takai T: Deletion of Fcγ receptor IIB renders H-2b mice susceptible to collagen-induced arthritis. J Exp Med 1999, 189:187–194
17. Jensen WA, Marschner S, Ott VL, Cambier JC. FcγRIIB-mediated inhibition of T-cell receptor signal transduction involves the phosphorylation of SH2-containing inositol 5-phosphatase (SHIP), dephosphorylation of the linker of activated T-cells (LAT) and inhibition of calcium mobilization. Biochem Soc Trans 2001, 29:840–846
18. Engelhardt W, Gorczytza H, Butterweck A, Monkemann H, Frey J: Structural requirements of the cytoplasmic domains of the human macrophage Fcγ receptor IIa and B cell Fcγ receptor IIb2 for the endocytosis of immune complexes. Eur J Immunol 1991, 21:2227–2238
19. Miettinen HM, Rose JK, Mellman L: Fc receptor isoforms exhibit distinct abilities for coated pit localisation as a result of cytoplasmatic domain heterogeneity. Cell 1989, 58:317–327
20. Cassard S, Choquet D, Fridman WH, Bonnerot C: Regulation of ITAM signaling by specific sequences in Ig-β B cell antigen receptor subunit. J Biol Chem 1996, 271:23786–23791
21. Ioan-Facsinay A, de Kimpe SJ, Hellwig SM, van Lent PL, Hofhuis FM, van Ojik HH, Sedlik C, da Silveira SA, Gerber J, de Jong YF, Roozendaal R, Aarden LA, van den Berg WB, Saito T, Mosser D, Amigorena S, Izui S, van Ommen GJ, van Vugt M, van de Winkel JG, Verbeek JS: FcγRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity 2002, 16:391–402
22. Hazenbos WL, Heijnen IA, Meyer D, Hofhuis FM, Renardel de Lavalette CR, Schmidt RE, Capel PJ, van de Winkel JG, Gessner JE, van den Berg TK, Verbeek JS: Murine IgG1 complexes trigger immune effector functions predominantly via FcγRIII (CD16). J Immunol 1998, 161:3026–3032
23. van Lent PL, van den Bersselaar LA, van den Hoek AE, van de Loo AA, van den Berg WB: Cationic immune complex arthritis in mice: a new model. Synergistic effect of complement and interleukin-1. Am J Pathol 1992, 140:1451–1461
24. Lens JW, van den Berg WB, van de Putte LBA: Quantitation of arthritis by ^{99m}Tc-uptake measurements in the mouse knee joint in inflammation scores. Agents Actions 1984, 14:723–728
25. Goebeler M, Roth J, Henseleit U, Sunderkotter C, Sorg C: Expression and complex assembly of calcium-binding proteins MRP8 and MRP14 during differentiation of murine myelomonocytic cells. J Leukoc Biol 1993, 53:11–18
26. Singer II, Kawka DW, Bayne EK, Donatelli SA, Weidner JR, Williams HR, Ayala JM, Mumford RA, Lark MW, Glant TT: VDIPEN, a metalloproteinase-generated neoepitope, is induced and immunolocalised in articular cartilage during inflammatory arthritis. J Clin Invest 1995, 95:2178–2186
27. van Meurs JBJ, van Lent PLEM, Holthuysen AEM, Singer II, Bayne EK, van den Berg WB: Kinetics of aggrecanase and metalloproteinase induced neoepitopes in various stages of cartilage destruction in murine arthritis. Arthritis Rheum 1999, 42:1128–1139
28. Lynch RG: Regulatory roles for FcγRIII (CD16) and FcγRII (CD32) in the development of T- and B-lineage lymphoid cells. J Leukoc Biol 2000, 67:279–284
29. van Lent PL, Dekker C, Mosterd J, van den Bersselaar L, van den Berg WB: Allergic arthritis induced by cationic proteins: role of molecular weight. Immunology 1989, 67:447–452

30. Azeredo da Silveira S, Kikuchi S, Fossati-Jimack L, Moll T, Saito T, Verbeek JS, Botto M, Walport MJ, Carroll M, Izui S: Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high-affinity anti-erythrocyte autoantibody. J Exp Med 2002, 195:665–672
31. Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, Takahashi K, Holers VM, Walport M, Gerard C, Ezekowitz A, Carroll MC, Brenner M, Weissleder R, Verbeek JS, Duchatelle V, Degott C, Benoist C, Mathis D: Arthritis critically dependent on innate immune system players. Immunity 2002, 16:157–168
32. Baumann U, Kohl J, Tschernig T, Schwerter-Strumpf K, Verbeek JS, Schmidt RE, Gessner JE: A codominant role of FcγRI/III and C5aR in the reverse Arthus reaction. J Immunol 2000, 164:1065–1070
33. Shushakova N, Skokowa J, Schulman J, Baumann U, Zwirner J, Schmidt RE, Gessner JE: C5a anaphylatoxin is a major regulator of activating versus inhibitory FcγRs in immune complex-induced lung disease. J Clin Invest 2002, 110:1823–1830
34. Hunziker W, Mellman I: Expression of macrophage-lymphocyte Fc receptors in Madin-Darby canine kidney cells: polarity and transcytosis differ for isoforms with or without coated pit localisation domains. J Cell Biol 1989, 109:3291–3302
35. Daeron M, Malbec O, Latour S, Bonnerot C, Segal DM, Fridman WH: Distinct intracytoplasmic sequences are required for endocytosis and phagocytosis via murine FcγRII in mast cells. Int Immunol 1993, 5:1393–1401
36. Schiller C, Janssen-Graalfs I, Baumann U, Schwerter-Strumpf K, Izui S, Takai T, Schmidt RE, Gessner JE: Mouse FcγRII is a negative regulator of FcγRIII in IgG immune complex-triggered inflammation but not in autoantibody-induced hermolysis. Eur J Immunol 2000, 30:481–490
37. van Lent PLEM, van den Bersselaar LAM, Holthuysen AEM, van Rooijen N, van de Putte LBA, van den Berg WB: Phagocytic lining cells in experimentally arthritis: downregulation of synovitis by CL_2MDP-liposomes. Rheumatol Int 1994, 13:221–228
38. Ramos-Ruiz R, Bernabeu C, Ariza A, Fernandez JM, Larraga V, Lopez-Bote JP: Arthritis transferred by cells derived from pre-inflammatory rat synovium. J Autoimmunity 1992, 5:93–106
39. Ehlers MRW: CR3: a general purpose adhesion-recognition receptor essential for innate immunity. Microbes Infect 2000, 2:289–294
40. Nielsen CH, Leslie RG: Complement's participation in acquired immunity. J Leukoc Biol 2002, 72:249–261
41. van Meurs JBJ, van Lent PLEM, Singer II, Bayne EK, van de Loo FAJ, van den Berg WB: IL-1ra prevents expression of the metalloproteinase-generated neoepitope VDIPEN in antigen-induced arthritis. Arthritis Rheum 1998, 41:667–656
42. Cawston T: Matrix metalloproteinases and TIMPS: properties and implications for the rheumatic diseases. Mol Med Today 1998, 4:130–137
43. van Meurs J, van Lent P, Holthuysen A, Lambrou D, Bayne E, Singer I, van den Berg W: Active matrix metalloproteinases are present in cartilage during immune complex-mediated arthritis: a pivotal role for stromelysin-1 in cartilage destruction. J Immunol 1999, 163:5633–5639
44. van Lent PL, Nabbe K, Blom AB, Holthuysen AE, Sloetjes A, van de Putte LB, Verbeek S, van den Berg WB: Role of activatory FcγRI and FcγRIII and inhibitory FcγRII in inflammation and cartilage destruction during experimental antigen-induced arthritis. Am J Pathol 2001, 159:2309–2320
45. Unemori EN, Bair MJ, Bauer EA, Amento EP: Stromelysin expression regulates collagenase activation in human fibroblasts. Dissociable control of two metalloproteinases by interferon-γ. J Biol Chem 1991, 266:23477–23482
46. Verheijen JH, Nieuwenbroek NM, Beekman B, Hanemaaijer R, Verspaget HW, Ronday HK, Bakker AH. Modified proenzymes as artificial substrates for proteolytic enzymes: colorimetric assay of bacterial collagenase and matrix metalloproteinase activity using modified pro-urokinase. Biochem J 1997, 323:603–609
47. Tomita T, Nakase T, Kaneko M, Shi K, Takahi K, Ochi T, Yoshikawa H: Expression of extracellular matrix metalloproteinase inducer and enhancement of the production of matrix metalloproteinases in rheumatoid arthritis. Arthritis Rheum 2002, 46:373–378
48. Yoon SO, Park SJ, Yoon SY, Yun CH, Chung AS: Sustained production of H_2O_2 activates pro-matrix metalloproteinase-2 through receptor tyrosine kinases/phosphatidylinositol 3-kinase/NF-κB pathway. J Biol Chem 2002, 277:30271–30282
49. Grage-Griebenow E, Flad HD, Ernst M, Bzowska M, Skrzeczynska J, Pryjma J: Human MO subsets as defined by expression of CD64 and CD16 differ in phagocytic activity and generation of oxygen intermediates. Immunobiology 2000, 202:42–50