High LDL-C levels attenuate onset of inflammation and cartilage destruction in antigen-induced arthritis

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Abstract

Objective

In this study, we used hypercholesterolaemic apolipoprotein E-deficient (Apoe$^{-/-}$) mice to investigate LDL/oxLDL effect on synovial inflammation and cartilage destruction during antigen-induced arthritis (AIA). Further, as macrophage FcγRs are crucial to immune complex-mediated AIA, we investigated in vitro the effects of high cholesterol levels on the expression of FcγRs on macrophages.

Methods

AIA was induced by intra-articular injection of mBSA into knee joints of immunised Apoe$^{-/-}$ and wild type (WT) control mice. Joint swelling was measured by uptake of ^{99m}Tc pertechnetate (^{99m}Tc). Joint inflammation and cartilage destruction were assessed by histology. Anti-mBSA IgGs were measured by ELISA and specific T-cell response by lymphocyte stimulation test. Upon oxLDL stimulation of WT macrophages, protein levels of FcγRs were measured by flow cytometry.

Results

Local induction of AIA resulted in less joint swelling, synovial infiltrate and exudate in the joint cavity in Apoe$^{-/-}$ mice compared to WT controls, even though both their humoral and adaptive immune response were comparable. Whereas Apoe deficiency alone did not affect macrophage expression of FcγRs, oxLDL sharply reduced the protein levels of activating FcγRs, crucial in mediating cartilage damage. In agreement with the reduced inflammation in Apoe$^{-/-}$ mice, we observed decreased MMP activity and destruction in the articular cartilage.

Conclusion

Taken together, our findings suggest that high levels of LDL/oxLDL during inflammation, dampen the initiation and chronicity of joint inflammation and cartilage destruction in AIA by regulating macrophage FcγR expression.

Key words

high LDL-C levels, oxLDL, macrophages, Fcγ receptors, cartilage destruction

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects 1–2% of the world population and is characterised by severe joint inflammation and destruction (1). RA is largely driven by intra-articular immune complexes (ICs), consisting of IgG class immunoglobulins together with their cognate antigen, which predominantly interact with macrophages via binding to Fcγ receptors (FcγRs) (2, 3). Previous studies have shown that synovial macrophages are crucial in the onset and propagation of experimental IC-mediated arthritis (4). In mice, macrophages express four subtypes of FcγRs (FcγRI, FcγRIIb, FcγRIII, FcγRIV), which differ in their affinity for the various IgG isotypes as well as in their function (5). FcγRI, FcγRIII and FcγRIV induce cell activation upon binding with ICs, while FcγRIIb inhibits cell activation and is involved in IC removal (6). Skewing of the FcγR balance on the surface of synovial macrophages towards activating FcγRs strongly increases the severity of joint inflammation and destruction (7). We showed that FcγRI plays a predominant role in mediating cartilage destruction and chondrocyte death via activation of matrix metalloproteases (MMPs) (8). On the other hand, mice lacking FcγRIIb showed increased inflammation and cartilage destruction, thus confirming the inhibiting effects of FcγRIIb (9, 10). Moreover, mice lacking FcγRI, IIb and III showed increased IC retention and inflammation, suggesting an additional pathological role for FcγRIV in this experimental model (11). RA is often associated with atherosclerosis, characterised by high levels of low-density lipoprotein cholesterol (LDL-C) (12, 13). Early RA is marked by increased serum levels of total cholesterol (TC) and LDL-C (14, 15). In contrast, active RA and chronic inflammation are accompanied by a reduction of TC and LDL-C serum levels (16, 17), although the clinical effect of high LDL-C levels in the initiation and progression of RA is still a matter of debate. Hence, we set out to determine how high systemic LDL-C levels modulate the onset and progression of disease. During inflammation, LDL-C levels in the joint resemble those in the blood (18), implying that people with high circulating LDL-C levels are subjected to have higher levels of LDL-C present in their joints. In an inflamed joint, IC-mediated cell activation leads to the production of reactive oxygen species (ROS) and results in the oxidation of LDL-C, thereby forming oxLDL, which is taken up by macrophages via scavenger receptors CD36, SR-A and LOX-1 (19) leading to cell activation. Previous studies have shown contradictory results of high cholesterol levels on the development of arthritis (20-22). However, the effects of high cholesterol levels on the expression of the immune-regulating FcγRs and their interplay with ICs remains to be elucidated. Therefore, in the present study we investigated the effects of high LDL-C levels on the development of cartilage destruction during experimental RA by induction of the IC-driven antigen-induced arthritis (AIA) model in Apolipoprotein E deficient (*Apoe*$^{-/-}$) mice, which spontaneously develop high LDL-C levels, and their wild type (WT) controls. Furthermore, as FcγRs are crucial to IC-mediated stimulation, we deepened our understanding of the effects of high cholesterol levels on the expression of FcγRs on macrophages.

Methods

Animals

Apoe$^{-/-}$ mice (JAX strain) were obtained from the Charles River Laboratory. Wild type C57Bl/6J mice were used as controls. All mice (10 mice/group) were housed in filter-top cages and received a standard chow diet and acidified water *ad libitum*. Male mice between 10–12 weeks were used in all the experiments, which were performed in accordance with the Dutch regulations and guidelines for care and use of laboratory animals. All animal studies were approved by the Radboud University's Animal Experiment Committee, Nijmegen-the Netherlands (RU-DEC 2014-191).

Induction of antigen-induced arthritis

Mice were immunised with 100 μg/mL of methylated bovine serum albumin (mBSA; Sigma-Aldrich) as previously

described (8). Three weeks after immunisation, arthritis was locally induced by intra-articular injection of 60 μg of mBSA in 6 μl saline in the knee joint.

^{99m}Tc pertechnetate uptake measurement

Joint swelling was measured by ^{99m}Tc pertechnetate uptake (^{99m}Tc) in the knee joint and scored as previously described (23).

Histology

Total knee joints were dissected, fixed in phosphate-buffered formalin (pH 7.4), decalcified in ethyilenediaminetetraacetic acid (EDTA) and subsequently embedded in paraffin. Coronal sections of 7 μm representing the whole joint were stained with haematoxylin & eosin (H&E) or Safranin O (SafO). See Supplementary material for the scoring of histological parameters.

Determination of cholesterol levels and IgG titres in serum

Total cholesterol, LDL-C and high-density lipoprotein cholesterol (HDL-C) levels were determined in the serum prior to induction of AIA in naive *Apoe*$^{-/-}$ mice and WT controls (3 mice/group), and at day 21 after AIA (10 mice/group). Values were calculated based on the Friedewald formula (24). The production of anti-mBSA specific antibodies (total IgG, IgG1, IgG2a and IgG2b) was determined by enzyme-linked immunosorbent assay (ELISA) as previously described (11).

Lymphocyte stimulation test

Spleens were collected (4 mice/group) at day 21 after AIA induction and homogenised through a cell strainer. Erythrocytes were lysed with lysis buffer (155 mM NH_4Cl, 12 mM $KHCO_3$, 0.1 mM ethylenediaminetetraacetic acid, pH 7.3). Cells were seeded into flasks and after 1h at 37 °C non-adherent cells were harvested and seeded into 96-well plates (1×10^5 cells/well). Cultures were maintained for 4 days in presence of 2-fold serial dilution of mBSA (starting with 25μg/mL) and for the last 16 hours ^{3}H-Thymidine was added. Its incorporation was determined as a measure of T-cell proliferation.

Luminex

Levels of cytokines and chemokines were measured in serum samples using Luminex multyanalyte technology and multiplex cytokine kits (Milliplex; Millipore), which sensitivity was <1pg/mL.

Culture of macrophages

Macrophages were differentiated from bone marrow-derived cells (BMDCs), previously isolated from naive WT and *Apoe*$^{-/-}$ mice by flushing the marrow cavity with DMEM using a syringe. BMDCs were differentiated into macrophages by culturing them for 6 days in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 15 ng/mL recombinant mouse macrophage-colony stimulating factor (RmM-CSF, R&D Systems), 10% fetal calf serum (FCS, Thermo Scientific), 1mM pyruvate and 1% penicillin and streptomycin (P/S).

oxLDL preparation and stimulation of macrophages

LDL was isolated by single-spin density gradient ultracentrifugation from EDTA-treated blood from healthy volunteers and oxidised as previously described (25). Bone marrow-derived macrophages (BMDMs) were then stimulated with either 10 μg/mL oxLDL or LDL-C as control. This concentration was chosen to resemble the LDL-C levels *in vivo* (26).

Flow cytometry

BMDMs stimulated for 24h with LDL-C or oxLDL and their unstimulated controls were washed with PBS and scraped from 24-well plates by using 10mM EDTA/PBS. See Supplementary materials for the antibodies used to determine FcγR expression.

Immunohistochemistry

Immunostaining was performed on whole joint sections to detect the presence of the neo-epitope VDIPEN, which is exposed following aggrecan cleavage by matrix metalloproteases (MMPs). A specific antibody directed against the VDIPEN cleaved site was used (2.5 μg/mL) and Rabbit IgG was used as isotype control. The amount of staining present in the patellofemoral and tibiofemoral areas was quantified in three consecutive sections per knee joint using an arbitrary score on a scale from 0 to 3.

Statistics

Statistics were performed using Graph Pad Prism v. 5.0 (GraphPad Software Inc., San Diego, CA). Differences between the two groups were tested using a two-tailed Student's *t*-test for comparing parametric variables, Mann-Whitney test for non-parametric variables (*e.g.* histological score) and multiple comparisons were tested using One-way ANOVA followed by Bonferroni's multiple comparison test. *p*-values <0.05 were considered significant. Data are presented as the mean ±95% CI.

Results

Apoe$^{-/-}$ mice develop less inflammation after induction of AIA

First, we determined the effects of high cholesterol levels on joint inflammation after induction of AIA in knee joints of previously immunised *Apoe*$^{-/-}$ mice and their WT controls. As expected, serum levels of total cholesterol (TC) in *Apoe*$^{-/-}$ mice were significantly higher than in WT controls, mainly due to a sharp increase of LDL-C levels. Of note, the induction of AIA in *Apoe*$^{-/-}$ mice reduced the serum levels of TC, as result of decreased LDL-C and HDL-C compared to naive mice (Fig. 1A). *Apoe*$^{-/-}$ mice showed significantly decreased ^{99m}Tc uptake as readout for joint swelling at days 1, 3, and 7 after induction of AIA as compared to WT controls (a reduction of 21%, 17% and 18%, respectively). However, at day 14 after induction, joint swelling was strongly reduced in both strains and the difference was lost (Fig. 1B). Underlining a difference in the early inflammatory response, in histology we observed a significant reduction in both the infiltrate and exudate in the knee joints of *Apoe*$^{-/-}$ mice (22% and 44% lower, respectively) at day 21 after induction (Fig. 1C-D). To determine whether *Apoe*$^{-/-}$ mice had a basal difference in synovial cellularity, we additionally scored contralateral control joints. However, no differences were found in infiltrate (Fig. 1E), whereas exudate was absent in these naive knee joints.

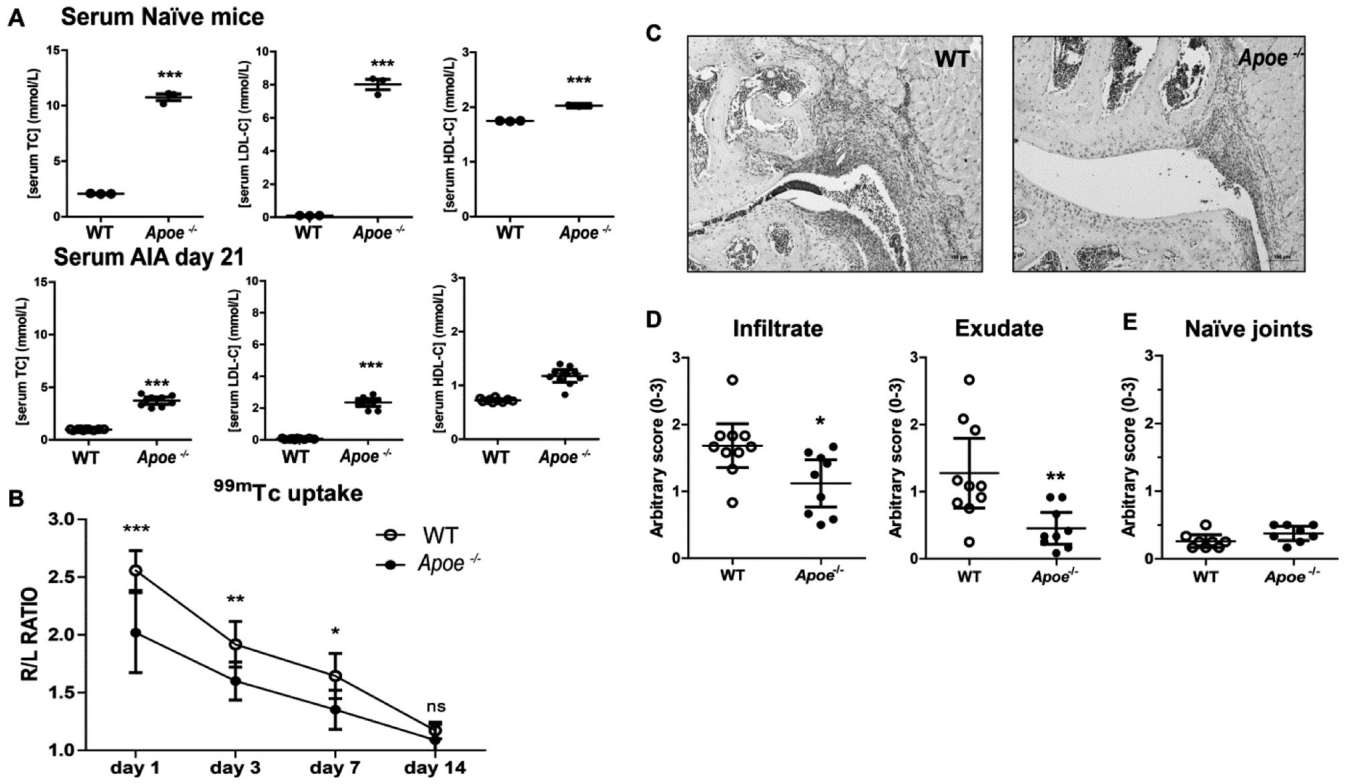

Fig. 1. *Apoe*$^{-/-}$ mice develop less joint inflammation during AIA.
A: The levels of total cholesterol (TC), LDL-C and HDL-C were determined in the serum of WT and *Apoe*$^{-/-}$ naïve and arthritic mice at day 21 AIA. Naïve *Apoe*$^{-/-}$ mice show significantly higher levels of TC than WT controls, mainly due to higher LDL-C. Notably, after AIA induction TC and LDL-C levels decreased, yet they were 3.8 times higher in *Apoe*$^{-/-}$ mice when compared to WT controls. Such increase was determined by higher LDL-C levels rather than HDL-C (55 *vs*. 1.6 fold higher, respectively). Horizontal and vertical lines represent the mean ±95% CI of 3 mice (naïve) or 10 mice (arthritic). **B**: R/L ratios of ^{99m}Tc uptake at day 1, 3, 7 and 14 after intra-articular injection of mBSA into the knee joints of mBSA-immunised *Apoe*$^{-/-}$ mice and their wild type (WT) controls. Note that WT mice display significantly higher joint swelling compared to *Apoe*$^{-/-}$ mice.
C: Representative images of cell infiltrate and exudate as determined by histology in *Apoe*$^{-/-}$ mice and WT controls at day 21.
D: Quantification of cell infiltrate and exudate showed that arthritic *Apoe*$^{-/-}$ mice had a significant reduction of infiltrate and exudate as compared to WT controls.
E: However, contralateral knee joints of WT and *Apoe*$^{-/-}$ mice had no signs of cell infiltration. Horizontal and vertical lines represent the mean ±95% CI of 8 mice (contralateral joints) or 10 mice (arthritic). (*p<0.05, **p<0.01, ***p<0.001). Original magnification, 100 x. ns = not significant'

The immune response is comparable in arthritic wild type and Apoe$^{-/-}$ *mice*
Because the induction of the AIA model is highly dependent on the formation of ICs that can bind to FcγRs, we determined whether *Apoe* deficiency influenced serum IgG titres. This would affect the amount and isotypic composition of ICs present in the joint, resulting in a less robust stimulation of FcγRs. However, we found that levels of total IgG, IgG1, IgG2a and IgG2b against mBSA in the serum were not significantly different between *Apoe*$^{-/-}$ and their WT controls at day 21 of AIA (Fig. 2A). Further, we determined the T-cell response against mBSA and found no significant differences (Fig. 2B), suggesting that the reduced inflammation observed in *Apoe*$^{-/-}$ mice is not due to an impaired humoral or adaptive immune response.

Apoe deficiency does not affect basal levels of FcγRs on macrophages
Since we observed no differences in the immune response or in the systemic production of pro-inflammatory cytokines (Fig. S1) that could explain the reduced inflammation, we next determined whether *Apoe* deficiency affected the expression of the receptors for ICs, the FcγRs, which are crucial in regulating the acute phase of AIA. Since macrophages play a pivotal role in the onset and propagation of disease, we compared the expression of FcγRs in *Apoe*$^{-/-}$ and WT BMDMs. However, the protein levels of FcγRs on the cell surface of *Apoe*$^{-/-}$ and WT BMDMs were not different as indicated by comparable MFI levels (FcγRI 44.2 *vs*. 44.6; FcγRIIb 43.9 *vs*. 43.8; FcγRIII 11.2 *vs*. 11.3; FcγRIV 32.1 *vs*. 32, respectively), suggesting that APOE is not involved in regulating basal FcγR expression (Fig. 3A-B).

oxLDL down-regulates the levels of FcγRI, II and IV on macrophages
APOE is important in lipid transportation and its absence spontaneously leads to high systemic levels of LDL-C, which is oxidised into oxLDL in an inflammatory *milieu*. As FcγR levels were similar in WT and *Apoe*$^{-/-}$ BMDMs, we next determined whether high LDL-C and oxLDL levels altered the expression of FcγRs. We stimulated WT BMDMs with either LDL-C or oxLDL for 24 hours *in vitro*, determined their lipid uptake by Oil Red O staining (Fig. 4A) and performed flow cytometry to assess the protein levels of FcγRs on the cell membrane. In contrast to LDL-C, oxLDL accumulated within the cells and strongly

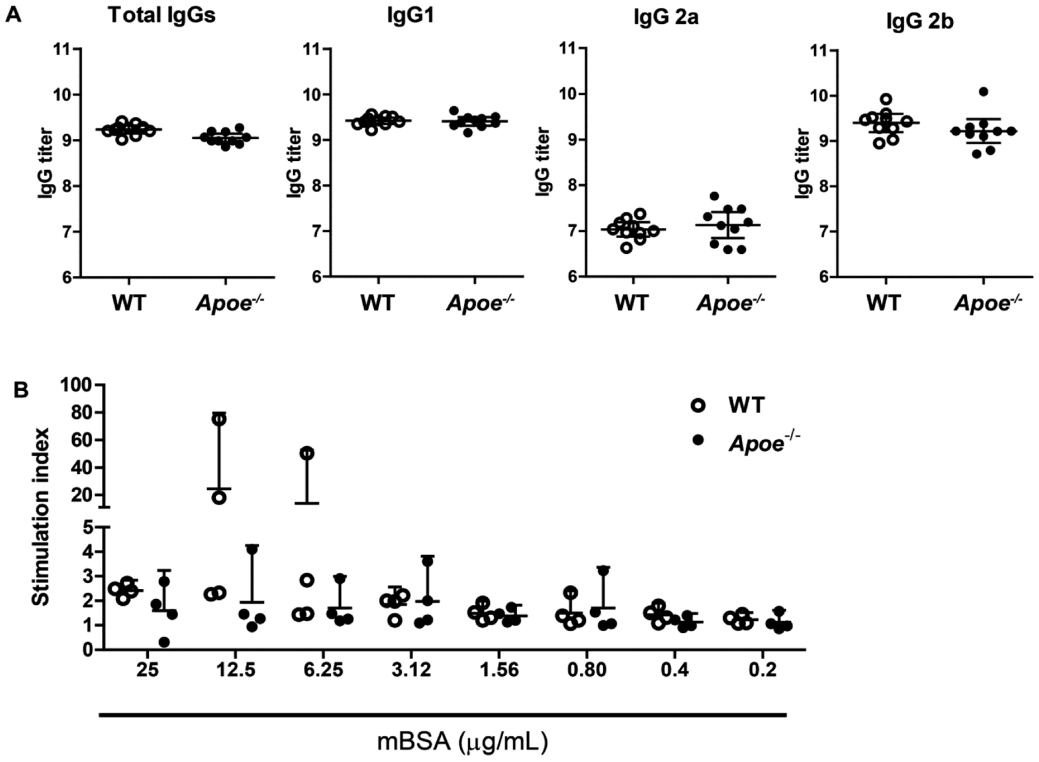

Fig. 2. The immune response is comparable between arthritic WT and *Apoe*$^{-/-}$ mice.
A: No significant differences were found in the production of anti-mBSA antibodies (total IgG, IgG1, IgG2a and IgG2b) between *Apoe*$^{-/-}$ mice and their WT controls (n=10 mice/group). Mean represents the two-log values using 50% of the maximal extinction as an endpoint.
B: The cellular immune response to mBSA, as determined by T-cell proliferation, was comparable between WT and *Apoe*$^{-/-}$ mice. Results are expressed as stimulation index (ratio of stimulation with/without antigen) (n=4 mice/group). Horizontal and vertical lines represent the mean ± 95% CI.

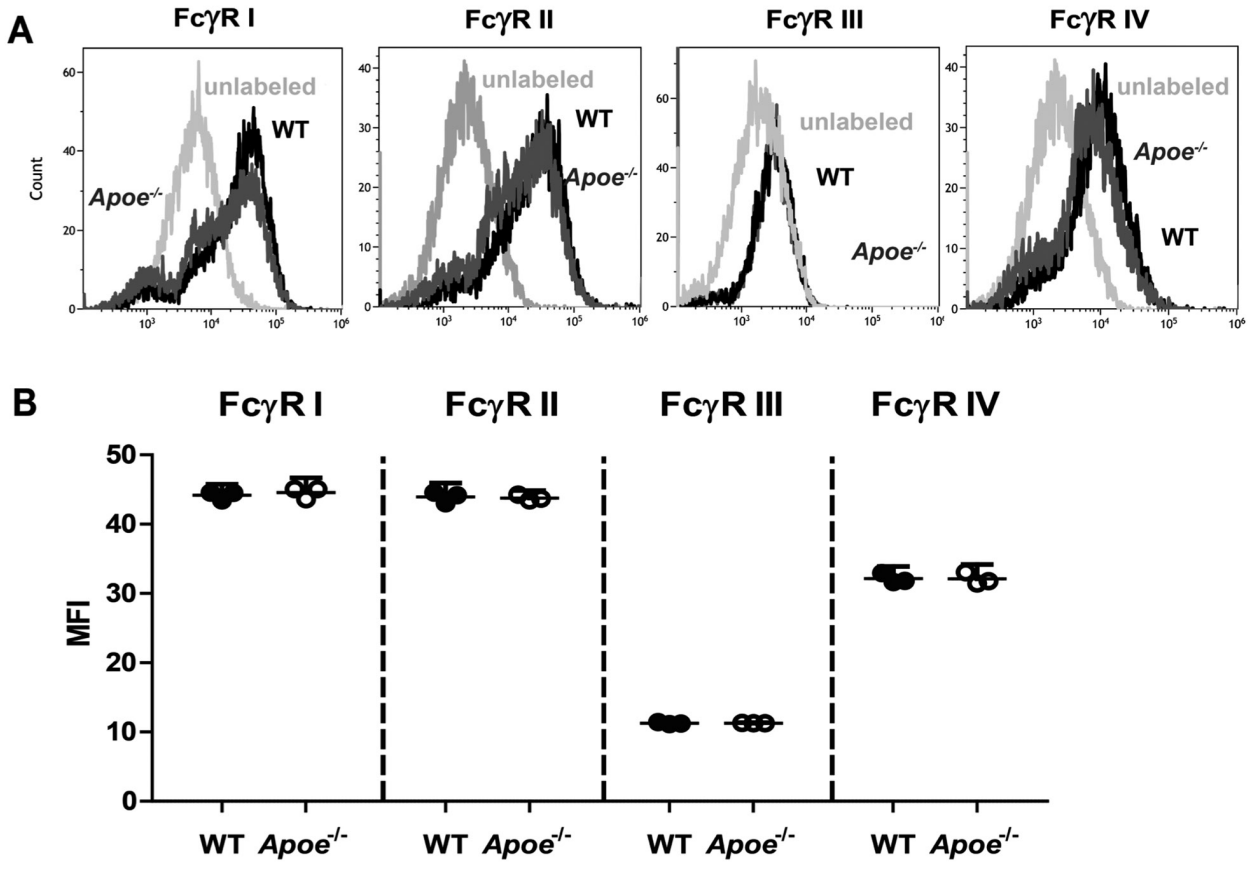

Fig. 3. *Apoe* deficiency does not affect FcγR levels on macrophages.
A: Histograms of WT and *Apoe*$^{-/-}$ BM- derived macrophages (BMDMs) showing the expression of the various Fcγ receptors (FcγRs).
B: Mean fluorescence intensity (MFI) show that WT and *Apoe*$^{-/-}$ BMDMs express comparable levels of FcγRs. One experiment representative of two independent experiments is shown.

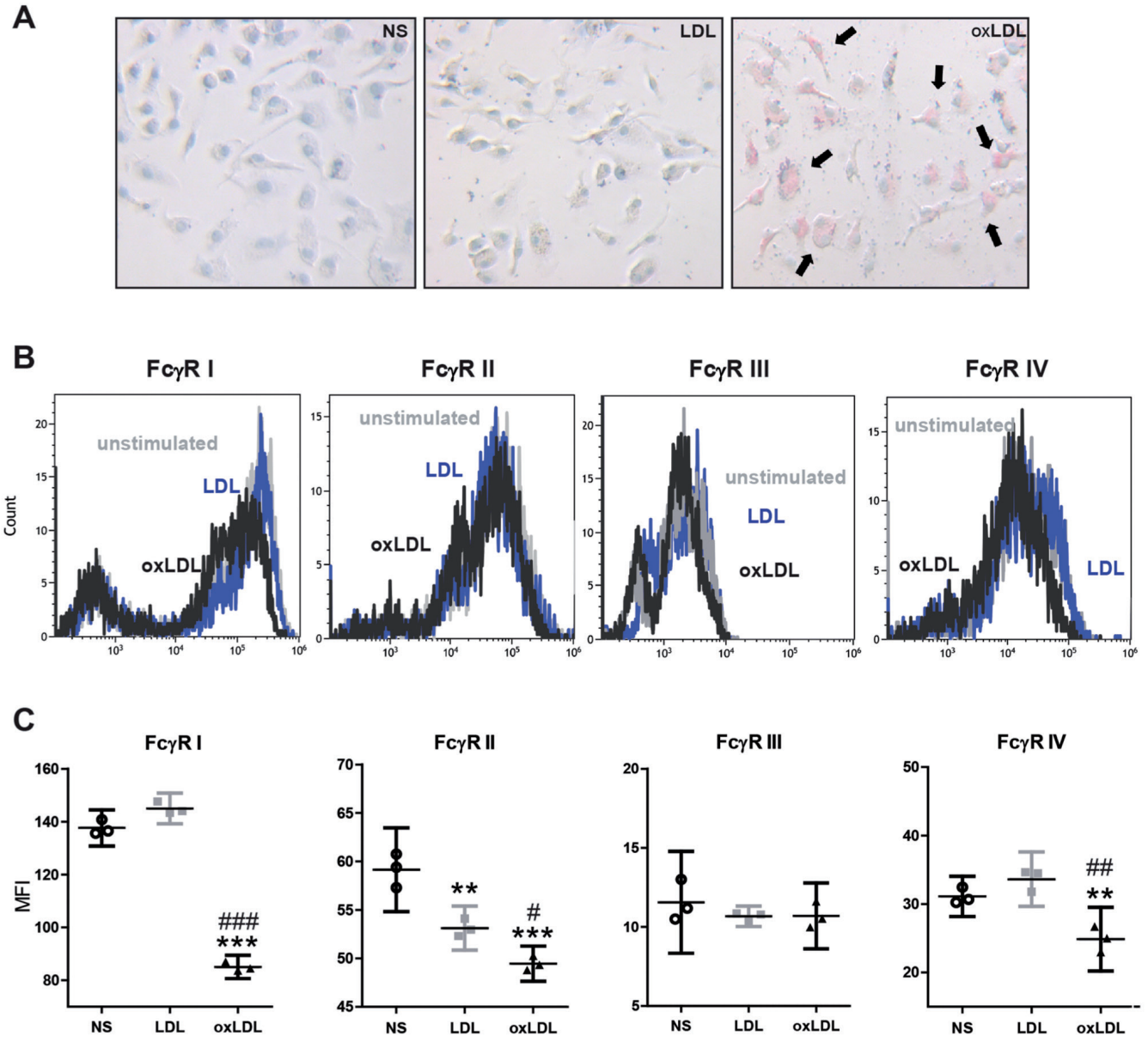

Fig. 4. oxLDL down-regulates the levels of FcγRs on macrophages.
WT bone marrow-derived macrophages (BMDMs) were cultured for 24 hours with or without 10 μg/mL LDL-C or oxLDL.
A: Oil Red O staining was used to investigate the uptake of LDL-C and oxLDL, which in contrast to LDL-C accumulated within the cell (black arrows). Original magnification, 100x.
B: Histograms of non-stimulated (NS), LDL-C or oxLDL-stimulated BMDMs are shown.
C: Mean fluorescence intensity (MFI) of FcγR expression in NS, LDL-C or oxLDL-stimulated macrophages. Both LDL-C and oxLDL significantly reduced the levels of FcγRIIb, while only oxLDL strongly reduced the activating FcγRI and IV (38% and 20% reduction, respectively). Conversely, the expression of FcγRIII remained unchanged. Horizontal and vertical lines represent the mean ±95% CI of one experiment representative of two independent experiments. *p<0.05, **p<0.01, ***p<0.001 *vs*. the NS control; # p<0.05 ## p<0.01, ### = p<0.001 *vs*. LDL-C stimulation.

lowered the expression of FcγRI and FcγIV (38% and 20%, respectively), while the expression of FcγRIII was not changed (Fig. 4B-C). Further, we found that both LDL-C and oxLDL, mildly reduced the expression of inhibiting FcγRIIb (10% and 16% lower, respectively). This dataset indicates that oxLDL leads to a strong reduction in the expression of activating FcγRI and IV on macrophages, thus underlining a role of oxLDL in modulating the innate immune response.

Apoe$^{-/-}$ mice show less cartilage destruction by reducing MMP activity in AIA

We previously showed that activating FcγRs mediate MMP activation, which is crucial for degradation of cartilage in AIA (8, 9). Because we found oxLDL to decrease the expression of FcγRI in macrophages, we next investigated whether high LDL-C levels during inflammation after induction of AIA in *Apoe$^{-/-}$* mice resulted in decreased MMP activity in the articular cartilage. We found a 52% reduction in the MMP-induced aggrecan neo-epitope VDIPEN in the cartilage of *Apoe$^{-/-}$* mice

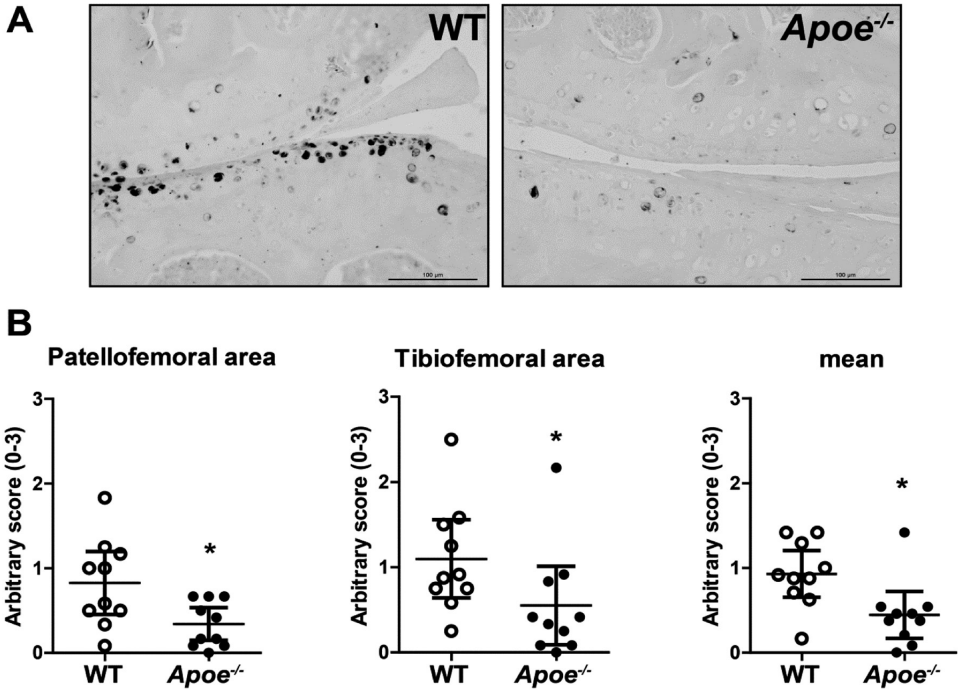

Fig. 5. *Apoe*$^{-/-}$ mice show reduced MMP activity during AIA.
A: Representative images showing MMP activity at day 21 after induction of AIA as determined by the presence of the neo-epitope VDIPEN with immunohistochemistry.
B: *Apoe*$^{-/-}$ mice showed a significant reduction of VDIPEN staining (44% lower), which was reduced by 63% in the patellofemoral area and by 60% in the tibiofemoral area. Horizontal and vertical lines represent the mean ±95% CI of 10 mice. Original magnification, 200x. *p<0.05

when compared to WT controls (mean arbitrary score 0.5±0.4 *vs*. 0.9±0.4, respectively) (Fig. 5A), with a significant reduction in VDIPEN neo-epitopes both in the patellofemoral area (0.3±0.3 *vs*. 0.8±0.5) and the tibiofemoral area (0.6±0.6 *vs*. 1±0.6) (Fig. 5B). In addition, we determined proteoglycan (PG) depletion and chondrocyte death as a further parameter for cartilage destruction. PG depletion and chondrocyte death were nearly absent in contralateral control knee joints (Fig. 6A and C) and no differences were observed between *Apoe*$^{-/-}$ mice and WT controls. In contrast, at day 21 of AIA, *Apoe*$^{-/-}$ mice showed a significant reduction both in PG content and chondrocyte death in the articular cartilage (20% and 24% reduction, respectively) when compared to WT controls (Fig. 6B and D). This indicates that high LDL-C levels by *Apoe* deficiency have a suppressive effect on cartilage destruction only in combination with inflammation. However, although we observed a difference in PG content and chondrocyte death, both groups of WT and *Apoe*$^{-/-}$ mice did not show signs of cartilage erosions yet at this time point.

Discussion

Several studies showed that dyslipidaemia is present in RA patients (27, 28). Active RA and chronic inflammation is accompanied by a reduction of total cholesterol (TC) and LDL-C serum levels (16, 17). In contrast, early RA is marked by increased serum levels of TC and LDL-C (14, 15). However, exactly how high levels of cholesterol influence onset and progression of RA remains poorly understood. In this study, we show that local induction of AIA in knee joints of hypercholesterolaemic *Apoe*$^{-/-}$ mice resulted in less joint inflammation and destruction of the articular cartilage during the course of arthritis. Moreover, we show that the mechanistic basis can be a decreased expression of FcγRs on macrophages.

In our study we investigated *Apoe*$^{-/-}$ mice on a normal diet that develop elevated LDL-C levels that resemble those found in humans. We previously showed that the synovial lining macrophages are crucial in regulating both the onset and progression of AIA (4, 29-31). Depletion of resident synovial macrophages prior to induction or during the course of arthritis completely prevented onset or continuation of arthritis (30, 31). This is likely because the onset of AIA is initiated by intra-articular injection of mBSA that interacts with synovial lining macrophages (32, 33) and the inflammation is driven by anti-mBSA antibodies forming ICs and their interaction with FcγRs on macrophages. In our study we found similar antibody titres of IgG subtypes against mBSA between *Apoe*$^{-/-}$ and WT mice. Apart from IgG mBSA-ICs, also IgG antibodies against oxLDL present in *Apoe*$^{-/-}$ mice, might contribute to disease activity (34). However, previous studies showed no association between these antibodies and development of atherosclerosis severity (35). This suggests that FcγR expression, rather than the level of ICs is altered by high cholesterol levels. In a naive joint FcγR expression on synovial lining macrophages is low (36). In contrast, in an inflamed synovium their expression is strongly enhanced (36) and the ratio between activating/inhibiting FcγRs expressed on macrophages contribute to accelerating inflammation. Systemic inflammation is raised in *Apoe*$^{-/-}$ mice (37). APOE is largely produced by macrophages and its production is strongly up-regulated by TGFβ and down-regulated by cytokines such as IL-1β, TNFα, IFNγ, and TLR4 ligands like LPS (38). APOE can modulate immune responses and act as an anti-inflammatory factor (39). However, we found that contralateral joints of *Apoe*$^{-/-}$ mice that were not injected with the antigen showed no signs of synovitis, indicating that in the absence of an IC trigger macrophages do not produce

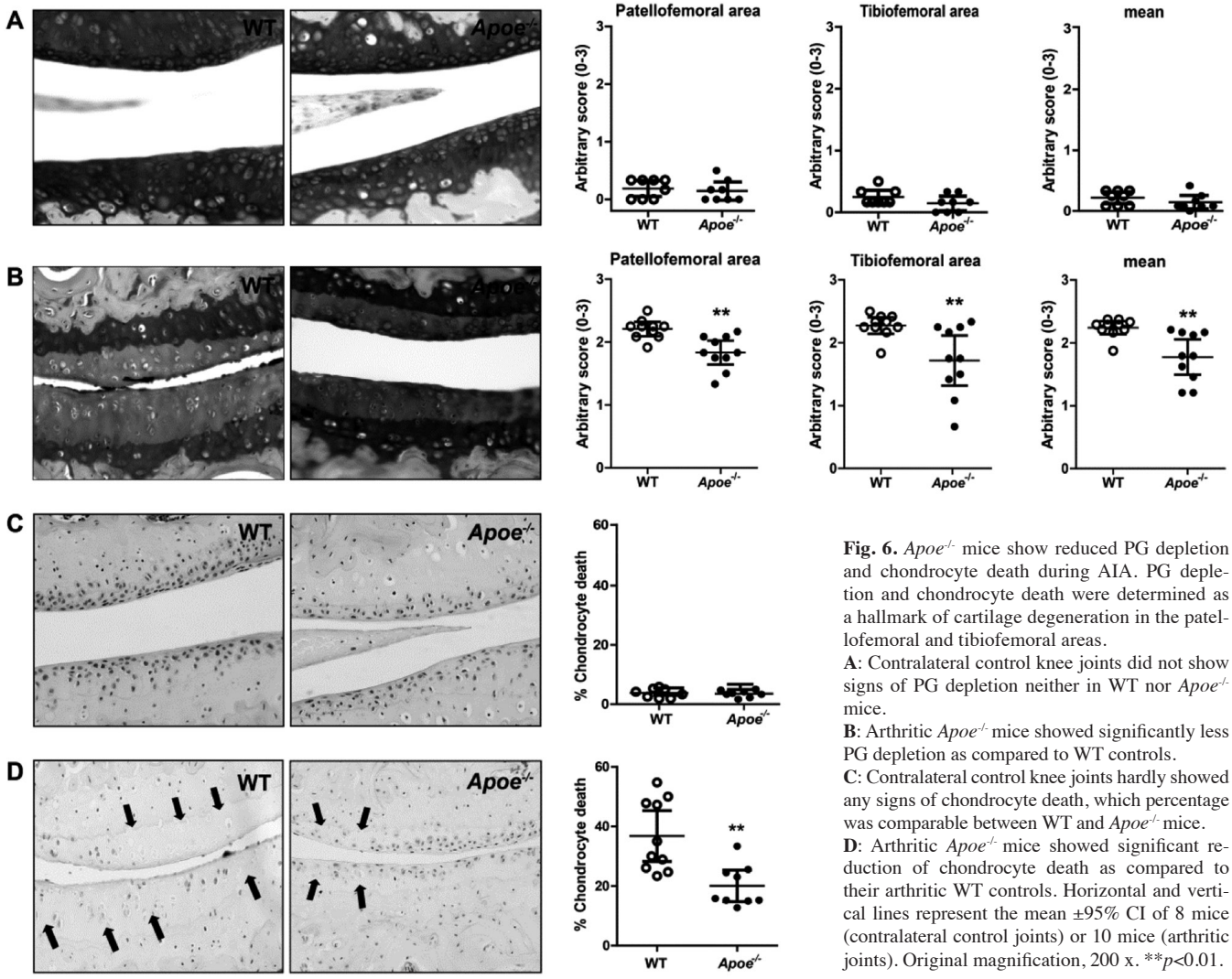

Fig. 6. *Apoe*$^{-/-}$ mice show reduced PG depletion and chondrocyte death during AIA. PG depletion and chondrocyte death were determined as a hallmark of cartilage degeneration in the patellofemoral and tibiofemoral areas.
A: Contralateral control knee joints did not show signs of PG depletion neither in WT nor *Apoe*$^{-/-}$ mice.
B: Arthritic *Apoe*$^{-/-}$ mice showed significantly less PG depletion as compared to WT controls.
C: Contralateral control knee joints hardly showed any signs of chondrocyte death, which percentage was comparable between WT and *Apoe*$^{-/-}$ mice.
D: Arthritic *Apoe*$^{-/-}$ mice showed significant reduction of chondrocyte death as compared to their arthritic WT controls. Horizontal and vertical lines represent the mean ±95% CI of 8 mice (contralateral control joints) or 10 mice (arthritic joints). Original magnification, 200 x. $^{**}p<0.01$.

inflammatory mediators and that high LDL-C levels alone are not sufficient to induce joint inflammation. In addition, the absence of APOE in macrophages did not affect their levels of FcγRs, indicating that its absence does not skew the balance of these receptors and therefore does not alter their sensitivity for ICs. Further, arthritic mice showed a similar immune response and comparable production of pro-inflammatory cytokines in the arthritic sera. However, we cannot exclude that APOE exerts *in vivo* other immune-modulatory functions that may be responsible for the down-regulation of FcγRs during inflammatory arthritis.

Interestingly, oxLDL but not LDL significantly down-regulated activating FcγRs, particularly FcγRI that is crucial in driving oxidative burst by activation of the dihydronicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, leading to massive ROS production and MMP activation (40). To a smaller extent, oxLDL reduced the expression of activating FcγRIV, which aggravates inflammation in the AIA model (11). Of note, the inhibiting FcγRIIb was also down-regulated upon both LDL and oxLDL. However, we assume that the stronger inhibition of activating FcγRI/IV overrules the modest reduction of inhibiting FcγRIIb, attenuating macrophage activation as a net result. LDL-C levels in the joint resemble those present in the blood (18). Upon entering an inflamed joint, LDL-C is oxidised by ROS into oxLDL and taken up by macrophages via scavenger receptors CD (36), SR-A and LOX-1 (19) and subsequently trafficked to lysosomes (41). OxLDL accumulation leads to massive cholesterol crystal formation, affecting lysosomal degradation of the FcγR-IC complex and recycling of FcγRs to cell membrane (42, 43).

Previous studies using *Apoe*$^{-/-}$ mice showed conflicting data on the effect of high LDL-C levels on the development of collagen-induced arthritis (CIA). Asquith *et al*. showed that *Apoe*$^{-/-}$ mice were resistant to develop CIA (20), whereas Postigo *et al*. showed increased severity of CIA (21). In the serum transfer-induced arthritis model (STIA), Archer *et al*. found that *Apoe*$^{-/-}$ mice developed more synovitis and a stronger infiltration of foam macrophages after repeated injections of K/BxN serum when fed a western type diet, which favored the formation of atherosclerotic lesions (22). These lesions may increase the systemic production of inflammatory mediators and have a different effect

on local joint inflammation, thus underlying the discrepancies with these findings. In our study, we did not observe foam macrophages in the synovium, probably due to the shorter duration of the model and administration of a normal diet. Interestingly, Archer *et al*. did not find differences in joint destruction (26). Although we did not corroborate the down-regulation of FcγR expression in the arthritic synovium, we find that oxLDL down-modulates macrophage FcγR expression, indicating it can be that its uptake by foam macrophages results in an even more pronounced decrease of FcγRs, thus reducing joint destruction.

Further, these controversial findings may be explained by the different genetic background of the *Apoe*$^{-/-}$ mice used. In a C57BL/6 (H2b) background, *Apoe*$^{-/-}$ mice were resistant against CIA (20, 44), whereas *Apoe*$^{-/-}$ mice in a B10.RIII background showed increased pathology in the CIA model (20). Previously, we showed using an IC-arthritis model that mice in a B10.RIII background developed more inflammation than C57BL/6 mice due to different kinetics of FcγR expression. IC stimulation of macrophages derived from the B10.RIII strain, which is susceptible to developing autoimmune diseases, elicited a stronger up-regulation of activating FcγRI as compared to macrophages derived from C57BL/6 non-susceptible strain, whereas the expression of the inhibiting FcγRIIb was strongly down-regulated. In contrast, the up-regulation of activating FcγR I and down-regulation of inhibiting FcγRIIb were more prolonged in B10.RIII-macrophages after IC stimulation, which skewed the balance towards activating FcγRs (45). As we find that oxLDL uptake by C57BL/6-macrophages efficiently down-regulated the activating FcγRs on their membrane, B10.RIII-macrophages may be less effective or even unable to regulate FcγRs after oxLDL uptake, which may explain the enhanced arthritis in *Apoe*$^{-/-}$ mice in a B10.RIII background. This study supports the hypothesis that previous controversial findings in *Apoe*$^{-/-}$ mice may be related to differences in the expression of FcγRs in the different strains.

Moreover, our findings underline the well known concept of the 'lipid paradox' in RA (46). Albeit dyslipidaemia increases the incidence of atherosclerosis in RA patients (47), previous studies observed no correlation between high LDL levels and the development of RA (46, 48). Furthermore, paradoxical outcomes have been described in RA patients concerning the effect of other metabolic and hormone-related factors. Adiponectin has been shown to act as a proinflammatory factor in the joints of RA patients, while they have an anti-atherogenic effect at the systemic level (49). Obesity, which is a major feature of the metabolic syndrome, is known to influence onset and progression of RA (50) and has been shown to reduce the risk of developing RA in men, but not in women (51). Interestingly, a study by Turesson *et al*. described that high LDL-C predispose to RA in women, but not in men, pointing at a role for sex-specific hormones in modulating the effects of lipid on RA pathogenesis (52). In this study, we only used male mice. Therefore, it remains to be elucidated which effects high LDL-C levels may have on female mice. Because we observed reduced pathology in male *Apoe*$^{-/-}$ mice this may be partially due to the exposure to sex-related hormones.

In addition to synovitis, high LDL-C levels by *Apoe* deficiency, in combination with inflammation reduced cartilage destruction. IC binding to activating FcγRs on macrophages, particularly FcγRI, leads to abundant ROS production and plays a central role in activating latent MMPs in the articular cartilage, leading to breakdown of glycosaminoglycans and chondrocyte death (9). A previous study showed that lack of FcγRI associated with reduced MMP activation and cartilage destruction during AIA (8). OxLDL strongly reduced FcγRI levels *in vitro*, suggesting that high LDL-C levels and their oxidation in the inflamed joint may be responsible for the reduction of MMP activation and cartilage destruction observed in this IC-driven arthritis model in *Apoe*$^{-/-}$ mice. The presence of tissue inhibitor of MMPs (TIMPs) is important in reducing the levels of MMPs (53). However, as in our *in vitro* experiments the expression of TIMPs was not increased (data not shown), we assume it is unlikely that increased TIMP expression as the result of high LDL or oxLDL levels may be the cause of decreased MMP activity as determined by VDIPEN staining.

Apart from lowering activating factors like FcγRI, oxLDL uptake by macrophages leads to activation of anabolic factors like TGFβ (25, 54). TGFβ can counteract the activity of pro-inflammatory cytokines like IL-1β (55), induce the expression of anti-inflammatory cytokines like IL-10 (56), up-regulate the inhibiting FcγRIIb and down-regulate the expression of the activating FcγRs (57).

Apart from affecting FcγR expression on macrophages, oxLDL may also target chondrocytes. However, we think it is unlikely that the reduced cartilage damage is mediated by a direct effect of oxLDL on FcγR regulation on chondrocytes, as the absence of either the activating or inhibiting FcγRs did not affect cartilage destruction in a model of experimental OA (58). Further, oxLDL can bind to LOX-1 on chondrocytes, increase the production of intracellular ROS and activate NF-kappaB, thus inducing a hypertrophic phenotype (59). Moreover, oxLDL binding to LOX-1 enhanced MMP3 production by chondrocytes (60), which may further contribute to cartilage pathology in RA. However, the data in our *in vivo* setting suggest that the suppressive effect induced by oxLDL on synovial macrophages may counterbalance oxLDL-mediated cartilage destruction as it was significantly decreased during the chronic phase of AIA. Collectively, our findings indicate that high levels of LDL/oxLDL by *Apoe* deficiency decreased inflammation and MMP-driven cartilage degeneration during onset and course of AIA, which is likely the result of oxLDL-driven FcγR down-regulation on synovial macrophages.

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