Toll-like Receptor 2 Controls Acute Immune Complex–Driven Arthritis in Mice by Regulating the Inhibitory Fcγ Receptor IIB

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Objective. Previous studies have demonstrated a protective role of Toll-like receptor 2 (TLR-2) and a proinflammatory function of TLR-4 during chronic T cell–driven arthritis. The involvement of TLRs in T cell–independent arthritic processes, however, remains unclear. This study was undertaken to determine the functional significance of TLR-2 and TLR-4 in T cell–independent immune complex–driven arthritis.

Methods. Serum-transfer arthritis was induced in wild-type and TLR-deficient mice by intraperitoneal injections of arthritogenic K/BxN mouse serum. Arthritis was assessed macroscopically and by histologic analysis. The influence of TLR-2 on macrophage cytokine profile, Fcγ receptor (FcγR) expression, and response to immune complexes was determined.

Results. While TLR-4, unexpectedly, did not play any significant role, TLR-2 deficiency accelerated the onset and markedly increased the severity of acute immune complex–driven arthritis in mice. TLR-2 deficiency resulted in a substantial increase in joint inflammation, bone erosion, and cartilage pathology, indicating a protective function of TLR-2 in passive FcγR-driven disease. Ex vivo study of the macrophage inflammatory phenotype revealed increased production of tumor necrosis factor α (TNFα) and interleukin-6 (IL-6) despite similar levels of IL-10, along with a significant increase in FcγR-specific response, in TLR-2−/− mouse macrophages early in the disease. Although distinct FcγR messenger RNA expression was not affected, cell surface protein expression of the inhibitory FcγRIIB in TLR-2−/− naive primary macrophages was specifically diminished, resulting in a higher proinflammatory response. Accordingly, TLR-2 stimulation specifically up-regulated FcγRIIB, but not the activating FcγR, on macrophages.

Conclusion. TLR-2 regulates acute immune complex–driven arthritis by controlling macrophage FcγR response. Our findings indicate that the protective role of TLR-2 is extended beyond its previously described role in promoting Treg cells during T cell–mediated arthritis.

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation and progressive cartilage and bone destruction in multiple joints. The pathogenesis of RA is grounded in a complex network of the components of innate and adaptive immune systems. Among the innate immune receptors, Toll-like receptors (TLRs) have recently gained attention due to a growing body of evidence indicating their involvement in RA.

TLRs are a family of pattern-recognition receptors evolved to recognize conserved microbe-associated molecular patterns. Increased expression of TLR-2, TLR-3, TLR-4, and TLR-7 in RA synovial tissue supports the idea that these receptors are relevant in the disease (1–3). Some TLRs can be activated by endoge-
nous “danger” molecules associated with inflammation and tissue destruction. Many of these endogenous TLR agonists have been found in the joints and serum of patients with RA and show a positive correlation with disease activity scores (1,4–8). TLR activation generally leads to a rapid and robust production of multiple inflammatory cytokines and matrix metalloproteinases (MMPs). In this regard, TLR-2 and TLR-4 have been shown to drive spontaneous production of tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) by intact RA synovial tissue explants cultured ex vivo (9,10).

In recent years, significant progress has been made in understanding the role of TLRs during arthritis. In vivo research has unraveled the role of TLR-4 in driving synovial inflammation and joint destruction in experimental models where T and B cell responses are actively involved (11,12). TLR-2, in contrast, was shown to inhibit T cell–mediated arthritis by promoting the suppressive function of Treg cells, thereby hampering the production of interferon-γ and IL-17 by T cells (9). Despite evidence of the involvement of TLR-2 and TLR-4 in regulating T cell–driven arthritis, the potential role and relevance of these receptors in T cell–independent arthritic processes remains poorly understood to date.

In the present study, we aimed to identify the functional significance of TLR-2 and TLR-4 in passive (T cell–independent) experimental arthritis induced by arthritogenic serum from K/BxN mice (13). The K/BxN serum contains autoantibodies directed against the ubiquitously expressed glucose-6-phosphate isomerase, forming IgG-containing immune complexes that activate another critical receptor family, Fcγ receptors (FcγR), on innate immune cells including monocytes and macrophages (13). Activation of FcγR by immune complexes has previously been shown to drive joint inflammation and severe cartilage destruction in experimental arthritis (14,15). Since RA is characterized by high levels of circulating IgG autoantibodies, activation of FcγR may play a major role and, therefore, the influence of TLRs on FcγR response to immune complexes will be of high relevance to understanding the pathogenesis of the disease.

In mice, FcγR comprise 4 classes: the activating receptors I, III, and IV, which among other effects induce cytokine production and oxidative burst upon triggering, and the inhibitory receptor IIb, which blocks the activating receptors through its intracellular immunoreceptor tyrosine–based inhibition motif (16). The balance between these receptors is a critical determinant of the net response to immune complexes. In addition, specific FcγR have been shown to play distinct roles in antibody-induced K/BxN serum-transfer arthritis. Moreover, FcγR IIB−/− mice manifest severe accelerated arthritis, whereas FcγRIII−/− mice develop an attenuated disease with delayed kinetics (17,18). One previous study demonstrated a crucial role of FcγR on bone marrow–derived rather than joint resident cells, and another study showed the involvement of macrophages in recognition of immune complexes and joint pathology in this model (17,19).

In the present study, we used the serum-transfer model to investigate the roles of TLR-2 and TLR-4 in the effector phase of arthritis devoid of T cell involvement and instead mediated via FcγR, and to study the regulation of FcγR expression and response in macrophages by TLR-2.

**MATERIALS AND METHODS**

**Induction of K/BxN serum–transfer arthritis.** C57BL/6 wild-type mice were purchased from Janvier, TLR-2−/− and TLR-4−/− mice on a C57BL/6 background were kindly provided by Professor S. Akira (Immunology Frontier Research Center, Osaka University, Osaka, Japan). Wild-type TLR-4−/− littermates were generated in house by crossing TLR-4−/− mice with wild-type C57BL/6 mice. Mice were housed in filter-top cages, and water and food were provided ad libitum. KRN T cell receptor–transgenic mice were a kind gift from Drs. C. Benoist and D. Mathis (Harvard Medical School, Boston, MA), and were maintained on a C57BL/6 background (K/B). Arthritic K/BxN mice were obtained by crossing K/B mice with NOD/Lt animals in the Nijmegen animal facility. Arthritis was induced by 2 intraperitoneal injections, 1 on day 0 and 1 on day 2, of 200 μl arthritogenic serum derived from arthritic K/BxN mice. The presence of endogenous TLR-2 and TLR-4 ligands in K/BxN serum was excluded using wild-type and TLR-deficient macrophages. Arthritis was macroscopically scored on a scale of 0–2 in each paw 3 times per week by 2 observers in a blinded manner. Animal studies were approved by the Animal Ethics Review Board of Radboud University Nijmegen.

**Isolation and culture of peritoneal and bone marrow–derived macrophages (BMMs).** Primary macrophages were isolated on days 2 and 4 after the first serum injection or from naive mice by lavage of peritoneal cavity using 10 ml of ice-cold phosphate buffered saline containing 10% fetal calf serum (FCS). Adherent cells were harvested and used for stimulations as described below. BMMs were generated by culturing bone marrow cells derived from femur in the presence of 15 ng/ml of macrophage colony-stimulating factor (R&D Systems) for 6 days. Cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco) supplemented with 10% FCS, 1 mM sodium pyruvate, and 50 mg/liter gentamicin.

**Stimulation of macrophages and measurement of cytokine levels.** To assess the macrophage cytokine profile during arthritis, mouse peritoneal macrophages were stimulated with phorbol myristate acetate (PMA; 50 ng/ml) and
ionomycin (1 µg/ml; Sigma) for 24 hours. To activate FcγRIIB, bovine serum albumin (BSA)-anti-BSA immune complexes were used. (BSA was obtained from Sigma.) Immune complexes were formed by incubating 500 µg/ml BSA with 100 µg/ml anti-BSA antibodies (Serotec) for 50 minutes at 37°C, then mixed with polymyxin B (10 µg/ml; Sigma) for 10 minutes before adding to the culture in order to block the effects of any potential endotoxin contamination. IL-1β (10 ng/ml; R&D Systems) and poly(I-C) (25 mg/ml; InvivoGen) stimulations were used as control. TLR-2 stimulation was achieved using Pam3Cys (1 and 10 ng/ml) and fibroblast-stimulating lipopeptides (both from EMC Microcollections). Immune complexes were formed by incubating 500 µg/ml anti-BSA antibodies (Serotec) for 50 minutes at 37°C, then mixed with polymyxin B (10 µg/ml; Sigma) for 24 hours. To activate FcγRIIB, bovine serum albumin (BSA)-anti-BSA immune complexes were used. (BSA was obtained from Sigma.) Immune complexes were formed by incubating 500 µg/ml BSA with 100 µg/ml anti-BSA antibodies (Serotec) for 50 minutes at 37°C, then mixed with polymyxin B (10 µg/ml; Sigma) for 10 minutes before adding to the culture in order to block the effects of any potential endotoxin contamination. IL-1β (10 ng/ml; R&D Systems) and poly(I-C) (25 mg/ml; InvivoGen) stimulations were used as control. TLR-2 stimulation was achieved using Pam3Cys (1 and 10 ng/ml) and fibroblast-stimulating lipopeptide 1 (FSL-1; 10 ng/ml) (both from EMC Microcollections). Cytokine concentrations in culture supernatants as well as mouse sera were determined using Milliplex cytokine assays according to the recommendations of the manufacturer (Millipore).

RNA isolation and quantitative polymerase chain reaction (qPCR). Synovial biopsy samples were obtained from the mouse knee joints using a 3-mm punch (Stiefel) on day 4 after the first serum injection or from naive mice. Total RNA isolation and real-time qPCR using SYBR Green Master Mix in an ABI Prism 7000 sequence detection system (Applied Biosystems) were performed as previously described (20). Primer sequences (forward and reverse, respectively) were as follows: for GAPDH (housekeeping gene), 5′-GGC-AAA-TTC-AAC-GGC-ACA-3′ and 5′-GGT-AGT-GGG-GTC-TG-3′; for IL-1β, 5′-GGG-CAG-AAT-ATC-AAC-CAA-GTG-ATA-3′ and 5′-GGG-TGC-CGT-CTT-TCA-TTA-CAC-AG-3′; for IL-6, 5′-GAA-GTG-GGC-GGC-TTA-ATT-ACA-CAT-G-3′ and 5′-ATT-GCC-ATT-GCA-CAA-CTC-TT-TCT-3′; for TNFα, 5′-CAG-ACC-CTC-ACA-CTCAGA-ACA-TCA-TCT-3′ and 5′-ATT-GCC-ATT-GCA-CAA-CTC-TT-TCT-3′; for MMP-3, 5′-GAG-TGC-TTA-GTG-ATA-ACC-C-3′ and 5′-TGA-AGC-CAC-GAA-CTA-GA-3′; for MMP-9, 5′-GAA-ACG-ACC-ACA-AGC-TCT-TCC-A-A′ and 5′-GAC-AGT-CAC-ACA-AGC-TCT-TCC-A-A′; for MMP-13, 5′-AGA-CCT-TGT-GTT-TGA-GGA-GCA-GTA-3′ and 5′-CTT-CAG-GAT-TCC-CGC-AAG-AG-3′; for FcγRIIIB, 5′-GAC-GIAA-CCG-GTT-ATT-ACA-AGC-ACA-3′ and 5′-GGA-CCG-TTG-ATT-ACA-AGC-ACA-3′; for FcγRIIIA, 5′-GGA-CCG-TTG-ATT-ACA-AGC-ACA-3′ and 5′-GGA-CCG-TTG-ATT-ACA-AGC-ACA-3′ and 5′-GGA-CCA-GCG-CAG-ACC-CCA-TAT-AG-3′; and for FcγRIIB, 5′-GAG-GTG-GGC-GTC-TCT-TCC-CAG-ACA-3′ and 5′-GGA-CCA-GCG-CAG-ACC-CCA-TAT-AG-3′. The threshold cycle (Ct) value of the gene of interest was corrected for that of GAPDH to obtain the ΔCt. Relative messenger RNA (mRNA) expression was calculated as 2−ΔCt.

Histologic analysis. Total ankle joints from mice were fixed in 4% formaldehyde for 4 days, then decalcified in 5% formic acid and embedded in paraffin. Tissue sections of 7 µm
were stained using hematoxylin and eosin to study inflammatory cell influx and chondrocyte death or using Safranin O to determine proteoglycan depletion, cartilage destruction, and bone erosion. Each parameter was scored on a scale of 0–3 by 2 observers in a blinded manner.

Fluorescence-activated cell sorting (FACS) analysis. Cell surface expression of distinct FcγR on macrophages was determined using allophycocyanin-conjugated anti-F4/80 (Serotec), Alexa Fluor 488-conjugated anti-FcγRI (X54-5/7.1; BD PharMingen), Alexa Fluor 647–conjugated anti-FcγRI (X54-5/7.1; BD PharMingen), Alexa Fluor 488–conjugated anti-FcγRIIB (clone K9.361 anti-Ly17.2) (kindly provided by Dr. S. Verbeek, Leiden University Medical Centre, Leiden, The Netherlands), fluorescein isothiocyanate–conjugated anti-FcγRII (clone 275003; R&D Systems), and Alexa Fluor 647–conjugated anti-FcγRII (clone 9E9) (a kind gift from Professor F. Nimmerjahn, University of Erlangen-Nuremberg, Erlangen, Germany). Cells were incubated with the antibodies described above or the appropriate isotype-matched control antibodies for 30 minutes at 4°C and analyzed on a FACSCalibur using CellQuest software (BD Biosciences PharMingen).

Statistical analysis. Group measures are expressed as the mean ± SEM. Statistical significance was assessed using the Mann-Whitney 2-tailed U test to compare 2 experimental groups and the Kruskal-Wallis test to compare more than 2 groups. Analysis was performed using GraphPad Prism 4.0 software. P values less than 0.05 were considered significant.

RESULTS

Significantly enhanced severity of immune complex–mediated arthritis in TLR-2−/−, but not TLR-4−/−, mice. To assess the involvement of TLR-2 and TLR-4 in T cell–independent immune complex–driven arthritis, passive K/BxN arthritis was induced in wild-type and TLR-deficient mice. TLR-4−/−, and especially TLR-2−/−, mice showed accelerated onset of the disease, reaching 100% incidence as early as 4 days after the first serum injection (Figure 1A). The mean time point of arthritis onset was 4.7, 2.8, and 3.3 days for the wild-type, TLR-2−/−, and TLR-4−/− groups, respectively. Interestingly, the disease severity score was markedly increased in TLR-2−/− mice during the acute phase (Figure 1B), indicating a negative regulatory function for TLR-2 during disease induction. The arthritis sever-
ity score was also significantly higher in TLR-2/−/− mice when only the mice with disease were considered, ruling out the influence of increased incidence.

In contrast to TLR-2, arthritis severity was, unexpectedly, not altered significantly by TLR-4 deficiency (Figure 1B). Although previous findings in other experimental models provide support for a proinflammatory role of TLR-4, repeating the study using TLR-4/−/− littermates confirmed that passive arthritis was not diminished, but rather tended to increase in TLR-4/−/− mice (Figure 1C). The considerable protective role of TLR-2 during acute immune complex–mediated arthritis was also reproduced in subsequent experiments regardless of the source of wild-type control mice (Figure 1D). In all experiments, disease scores during the resolution phase appeared not to be influenced by TLR-2 or TLR-4 deficiency (Figures 1B and C).

Potentiated inflammatory profile and FcγR-specific response of macrophages during passive arthritis upon TLR-2 deficiency. The processes underlying the increased arthritis severity observed in TLR-2/−/− mice were further investigated during the acute phase. To this end, the cytokine profile of macrophages as important mediators of K/BxN passive arthritis was determined ex vivo by nonspecific activation with PMA and ionomycin. TLR-2/−/− peritoneal macrophages isolated as early as day 2 of disease induction, i.e., prior to any significant change in arthritis scores, produced much higher levels of TNFα and IL-6 than did wild-type mouse macrophages (Figures 2A and B). Notably, production of the antiinflammatory cytokine IL-10 was not affected in TLR-2/−/− mouse macrophages (Figure 2C). Of great interest, the FcγR-specific proinflammatory response upon triggering with BSA–anti-BSA immune complexes was markedly increased in TLR-2/−/− mouse macrophages at this early time point (Figures 2A and B). The production of cytokines by macrophages stimulated with other agents, such as IL-1β and poly(I-C), was similar in wild-type and TLR-2/−/− cells (Figures 2A–C and data not shown), suggesting that the regulatory role of TLR-2 in macrophage proinflammatory response is specific to FcγR stimulation.

Figure 3. TLR-2 regulates joint inflammation and arthritic bone erosion in mice during passive immune complex–mediated arthritis. A, Synovial expression of proinflammatory genes on day 4 of serum-transfer arthritis in wild-type and TLR-2/−/− mice, as determined by quantitative polymerase chain reaction. The ΔCt values after correction for the housekeeping gene GAPDH were used to calculate relative mRNA expression (2−ΔΔCt). B and C, Histologic assessment of joint inflammation (B) and bone erosion (C), scored on a scale of 0–3. Sections of mouse ankle joints isolated on days 4 and 7 were stained with hematoxylin and eosin (H&E) to score joint inflammation and with Safranin O to score bone erosion. In A–C, bars show the mean ± SEM (n = 5 mice per group). * = P < 0.05; ** = P < 0.01, wild-type versus TLR-2/−/− mice, by Mann-Whitney U test. D, Representative images of H&E-stained sections (left) and Safranin O–stained sections (right) obtained from mice with arthritis on day 7, illustrating increased inflammation (arrows in left panels) and arthritic bone erosion (arrows in right panels) in TLR-2/−/− mice. JC = joint cavity; B = bone. Original magnification × 100. See Figure 2 for other definitions.

TLR-2 INHIBITS ACUTE IMMUNE COMPLEX-MEDIATED ARTHRITIS VIA FcγRIIB
The potentiated inflammatory profile and FcγR response of TLR-2 primary macrophages was clearly visible on day 4 of arthritis as well. The mean SEM stimulation index compared to medium control, e.g., for TNFα production, was 3.6 ± 0.3 in wild-type mice versus 13.8 ± 1.7 in TLR-2−/− mice upon PMA/ionomycin stimulation and 2.9 ± 0.4 in wild-type mice versus 56.3 ± 11.9 in TLR-2−/− mice upon FcγR stimulation (P < 0.05 for both stimulations). Production of IL-6 was also significantly increased upon stimulation with PMA/ionomycin or FcγR; however, the effect of IL-1 stimulation on IL-6, as well as TNFα, production remained unaltered by TLR-2 deficiency (data not shown). In addition, increased production of IL-6 along with the keratinocyte-derived chemokine in TLR-2−/− mice with passive arthritis was evident in serum at early stages (Figure 2D and data not shown). TNFα was undetectable in serum.

Regulation of joint inflammation and arthritic bone erosion by TLR-2 during passive immune complex–mediated arthritis in mice. The involvement of TLR-2 in joint pathology induced by T cell–independent immune complex–mediated arthritis was investigated locally. Synovial tissue from TLR-2−/− mice expressed higher transcript levels of mRNA for IL-1β, TNFα, and IL-6 than synovial tissue from wild-type mice (Figure 3A). K/BxN serum-transfer arthritis was accompanied by mild synovial inflammation and bone erosion in wild-type mice on day 4, which increased in time toward day 7 (Figures 3B and C). Importantly, TLR-2 deficiency resulted in a substantial increase in joint inflammation as well as bone pathology (Figures 3B and C). Figure 3D shows representative images of joint histopathology on day 7 in wild-type mice as compared to TLR-2−/− mice.

**TLR-2 protects against cartilage pathology during immune complex–mediated arthritis in mice.** Synovial expression of mRNA for the cartilage-degrading enzymes MMP-3 and MMP-9 was induced during serum-transfer arthritis and tended to be higher in TLR-2−/− mice (Figure 4A). MMP-13 expression was low in wild-type mice but increased significantly in TLR-2−/− mice (Figure 4A). Next, histologic analysis of several features of cartilage pathology, including matrix proteoglycan depletion, chondrocyte cell death, and
cartilage surface erosions, was performed. TLR-2−/− cartilage showed more severe proteoglycan depletion as evidenced by loss of red Safranin O staining, and higher degrees of chondrocyte death as represented by empty lacunae (Figures 4B and C). Only 2 of 5 TLR-2−/− mice showed signs of very mild cartilage surface erosions. Increased immune complex–driven cartilage pathology upon TLR-2 deficiency is illustrated in Figure 4D.

*Increase in FcγRIIB expression and negative regulation of FcγR response by TLR-2 in naive mouse primary macrophages.* Considering the notable protective role of TLR-2 in passive immune complex–mediated arthritis, we examined the involvement of TLR-2 in regulating the expression of activating and inhibitory FcγR. Quantitative PCR analysis showed that basal expression of mRNA transcripts for FcγR under naive conditions was not affected by TLR-2 deficiency in mouse peritoneal lavage cells or synovial tissue (Figure 5A and data not shown). However, measurement of cell surface expression of the 4 distinct FcγR classes revealed significant and specific reduction of FcγRIIB expression on TLR-2−/− cells from peritoneal lavage, while the activating receptors were unaltered (Figures 5B and C). Stimulation of peritoneal cells from naive nonarthritic mice with immune complexes resulted in higher concentrations of IL-6, TNFα, and IL-1β, but comparable IL-10 production, in TLR-2−/− mice versus wild-type mice (Figure 5D).

Since cells isolated from the mouse peritoneal cavity might potentially have been exposed to low concentrations of endogenous or gut-derived TLR agonists, we investigated FcγR expression and response in naive unstimulated mouse BMMs. A similar specific reduction in FcγRIIB, but not in FcγRI, III, or IV, was observed in Figure 5.
TLR-2−/− mouse BMMs as compared to wild-type mouse cells (Figure 6A). The production of cytokines in response to immune complexes was less potent in naive BMMs than in peritoneal cells, but nevertheless clearly confirmed the negative regulatory function of TLR-2 in macrophage FcγR response (Figure 6B).

Consistent with these observations, stimulation of TLR-2 on naive mouse BMMs induced a specific up-regulation of FcγRIIB in a dose-dependent manner, revealing a mean ± SEM of 39.0 ± 0.8% FcγRIIB cells at the highest ligand concentration used (10 ng/ml) as compared to 11.7 ± 2.8% in medium control (Figure 6C). Expression of the activating FcγR was, however, either not affected or very marginally affected (for FcγRI, 78.0 ± 4.2% versus 80.7 ± 1.1%; for FcγRIII, 7.2 ± 0.8% versus 8.3 ± 1.0%; and for FcγRIV, 7.9 ± 2.1% versus 11.5 ± 3.5% in medium control and after stimulation with 10 ng/ml TLR-2 agonist, respectively). Furthermore, induction of FcγRIIB expression was achieved by both Pam3Cys engaging a TLR-1/TLR-2 heterodimer and FSL-1 activating a TLR-2/TLR-6 functional heterodimer (Figure 6D). Taken together, these data indicate a negative regulatory function of TLR-2, in conjunction with either TLR-1 or TLR-6 as partner, in FcγR response through specific induction of the inhibitory FcγRIIB.

**DISCUSSION**

Considerable evidence has accumulated over the last decade to support the involvement of TLR-2 and TLR-4 in the pathogenesis of RA. Identification of specific RA-related processes to which these receptors contribute would help clarify the disease mechanisms involved and allow therapeutic interventions to be targeted to specific phases of the disease or specific subgroups of patients.

Importantly, TLR-2 activation has recently been shown to promote angiogenesis in RA synovial explants and to induce endothelial cell adhesion and invasion (21). Despite a tremendous amount of data indicating a proinflammatory and tissue-destructive role of TLR-4 in
various, mainly T cell–driven, experimental models, in vivo studies of the role of TLR-2 during arthritis are very limited. TLR-2 is responsible for acute arthritis directly induced by intraarticular injection of microbial components such as streptococcal cell wall fragments and zymosan, which are specifically recognized by this receptor (20,22,23). However, in experimental models devoid of direct microbial TLR triggering but with additional adaptive immune activation, the function of TLR-2 is likely to be different. Since TLR-2 is important for the optimal differentiation and function of Treg cells (24,25), TLR-2−/− mice develop a severe destructive disease when T cell immunity is involved (9).

This study was undertaken to unravel the as-yet largely unknown role of TLR-2 and TLR-4 in T cell–independent arthritic processes. Activation of FcγR during the effector phase of the disease is a relevant aspect in this context. In contrast to the active T cell–dependent K/BxN arthritis resulting from the KRN T cell receptor transgene, the FcγR-dependent serum-transfer model can be optimally induced in T cell–and B cell–deficient (RAG0/0) mice (13) and was therefore used in this study. Although serum-transfer arthritis can be augmented by external addition of KRN T cells, these autoreactive T cells are not present in non-KRN transgenic mice (26). The spleens of BL/6 mice with serum-transfer arthritis lack significant levels of Th17 cells, and the disease is independent of IL-17 in the absence of transferred KRN T cells (26). These characteristics enabled us to investigate the T cell/Th17–independent role of TLR-2 and TLR-4 in immune complex–mediated arthritis.

There is some controversy regarding the role of TLR-4 in passive immune complex–induced arthritis. TLR-4 mutant (C3H/HeJ) mice were shown to exhibit less severe arthritis during the chronic (resolution) phase of the K/BxN serum-transfer model than wild-type mice, while having a similar initiation phase (27). Two other studies using TLR-4 gene–deficient mice showed, however, either clinical scores and ankle inflammation similar to those seen in wild-type mice throughout the entire disease course (28), or a protected phenotype from the early induction phase (29). Consistent with the findings of Christianson et al (28), our study revealed that TLR-4 does not promote immune complex–mediated arthritis. In fact, TLR-4−/− mice tended to develop slightly more severe disease. The nonsignificant role of TLR-4 in serum-transfer arthritis is distinct from its evident role in other arthritis models, including collagen-induced arthritis, methylated BSA antigen–induced arthritis, and chronic streptococcal cell wall–induced arthritis, as well as the spontaneous arthritis in IL-1 receptor antagonist–deficient (IL-1Ra−/−) mice, all of which strongly rely on T cell activation and development of pathogenic Th17 cells (9,11,12,20,30–32). Hence, the data regarding the redundancy of TLR-4 in serum-transfer arthritis clarify its divergent role in distinct disease-related processes.

The interaction between TLRs and FcγR has been the subject of several studies. Immune complexes containing citrullinated fibrinogen have been shown to induce TNFα production in human and murine macrophages via concomitant engagement of TLR-4 and FcγR (33). A recent study showed that cotriggering of FcγRIIA amplified the cytokine response of human dendritic cells to TLR-2, TLR-4, and TLR-5 in order to promote Th17 differentiation (34). Considering the potential and robust inflammatory effects of both TLRs and FcγR, tight control of their response is desired. In fact, counterregulation of TLR response by FcγRIIB has been suggested before. In this context, FcγR ligation can inhibit the responses of TLR-2 and TLR-4 to their respective agonists via activation of the phosphatidylinositol 3-kinase/Akt pathway (35–37). However, it has so far been unclear whether TLRs also pursue a counteregulatory mechanism to control FcγR response. To our knowledge, this is the first study to show that TLR-2 indeed limits FcγR activation by immune complexes, a process with notable consequences for antibody-driven joint pathology.

TLR-2 deficiency exaggerated the inflammatory phenotype and FcγR response in primary macrophages at a very early stage of the disease. This resulted in increased expression of TNFα and IL-1β, for which relevant roles in this serum-induced model have been demonstrated (27,38). TLR-2 stimulation was found to specifically up-regulate the inhibitory FcγRIIB, which dampens the response of other FcγR. Accordingly, TLR-2−/− mice have reduced FcγRIIB expression on macrophages and exhibit a phenotype very similar to that previously observed in FcγRII−/− mice (17). Since TLR-2 has been reported to induce alternative macrophage activation via IL-4Ra and STAT-6 (39), expression of a variety of markers for alternatively activated macrophages including arginase 1, CD163, YMI1, and IL-1Ra and IL-1RII was analyzed along with markers for classically activated macrophages such as inducible nitric oxide synthase and CD86 (40). However, no evidence of reduced alternatively activated macrophages and skewing toward the classically activated macrophage phenotype was found in TLR-2−/− mice (data not shown).
It remains to be determined exactly how TLR-2 controls cell surface expression of FcγRIIB. Various endogenous TLR-2 agonists have been described in the context of inflammatory diseases such as RA; however, it is unclear whether these agonists are at play in our model. The observation that FcγRIIB expression is already altered in naive unstimulated BMMs differentiated from BM cells ex vivo tends to exclude an indirect effect of ligand-mediated TLR-2–induced cytokines modulating FcγRII expression. Extensive flow cytometric analyses showed similar proportions of FcγR-bearing myeloid blasts, granulocytes, and monocytes in the bone marrow of wild-type and TLR-2−/− mice with unaltered FcγRI expression (data not shown). However, the influence of TLR-2 deficiency on other mature FcγR-expressing cells, such as mast cells and neutrophils, that contribute to disease is not excluded. A previous study showed that triggering with IgG immune complexes causes direct physical association of TLR-4 with endocytic receptors targeting these receptors in the future.

The observation that FcγRIIb deficiency on other mature FcγR-expressing cells, such as mast cells and neutrophils, that contribute to disease is not excluded. A previous study showed that triggering with IgG immune complexes causes direct physical association of TLR-4 with endocytic receptors targeting these receptors in the future.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Abdollahi-Roodsaz had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Abdollahi-Roodsaz, Koenders, van de Loo, van den Berg.

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