

Destructive Role of Myeloid Differentiation Factor 88 and Protective Role of TRIF in Interleukin-17–Dependent Arthritis in Mice

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Objective. Increasing evidence indicates the involvement of Toll-like receptors (TLRs) in the progression of arthritis; however, the contribution of the two signaling pathways used by TLRs, which are mediated by myeloid differentiation factor 88 (MyD88) and TRIF, remains unclear. The objective of this study was to investigate the specific roles of MyD88 and TRIF in chronic experimental arthritis and the accompanying adaptive immune responses.

Methods. Chronic arthritis was induced in wild-type, MyD88^{-/-}, and *Trif*^{clps2} (TRIF^{-/-}) mice by repetitive intraarticular injections of streptococcal cell wall (SCW) fragments. SCW-specific T cell and B cell responses, joint swelling, and histopathologic changes were analyzed during chronic arthritis.

Results. Both MyD88 and TRIF pathways contributed to antigen-specific T cell proliferation and antibody production, with the MyD88 pathway playing the dominant role. The severity of joint swelling and synovial inflammation, as well as the histopathologic

damage to cartilage and bone, was strongly dependent on MyD88 signaling, whereas TRIF was redundant. MyD88 signaling was critical for the development of pathogenic T cell response (i.e., interleukin-17 [IL-17] production) in response to SCW antigen. Interestingly, when the T cell–dependent phase was prolonged, TRIF signaling appeared to down-regulate bone erosion, an effect accompanied by an inhibitory effect on IL-17 production.

Conclusion. This study reveals a central role of MyD88 and a counterregulatory function of TRIF in T cell–driven arthritis. The findings provide a rationale for a pathway-specific interference in order to block the pathogenic features and to preserve or stimulate the beneficial aspects of TLR signaling.

Rheumatoid arthritis (RA) is a chronic inflammatory, destructive disease of multiple joints that may lead to disability in patients with the progressive form. Accumulating evidence suggests the involvement of a recently identified family of receptors of the immune system, Toll-like receptors (TLRs), in inflammation and destruction driven by multiple cellular players in RA. Expression of TLRs 2, 3, 4, and 7 is enhanced in (early) RA synovial tissue (1,2), and exogenous as well as endogenous TLR agonists have been detected in the joints of patients with RA (3–7). TLRs generally exert proinflammatory and catabolic effects on nonimmune cells relevant to RA, such as fibroblasts and chondrocytes (8,9). Furthermore, TLR-2 and TLR-4 on immune cells, such as blood-derived mononuclear cells and dendritic cells, from RA patients are hyperresponsive to their respective ligands (2,10,11). Importantly, very recent reports implicate TLR-2 and TLR-4 activation in the spontaneous release of proinflammatory cytokines,

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including tumor necrosis factor α (TNF α) and interleukin-1 β (IL-1 β) by RA synovial tissue (12,13).

Some TLR agonists can profoundly enhance arthritis expression upon injection in vivo (12,14). In animal models of RA, TLRs, in particular TLR-4, drive the expression of inflammatory cytokines and determine the severity of joint inflammation and destruction (12,15). TLR-4 activation seems to mainly contribute to the chronic phase of arthritis, probably due to the presence of endogenous damage-associated agonists in this phase (12,16). In this regard, in arthritis induced by repetitive intraarticular injections of bacterial TLR ligands derived from streptococcal cell walls (SCWs), the TLR-2-dependent acute phase shifts toward a TLR-4-driven chronic phase in which TLR-4 determines the antigen-specific production of the pathogenic cytokine IL-17 (17). General inhibition of endosomal TLRs (TLR-3, TLR-7, and TLR-9) was recently reported to ameliorate murine arthritis as well (18).

Despite the great body of evidence indicating the involvement of TLRs in RA, the specific roles and the relative contribution of TLR signaling pathways in the disease are not well understood. TLR signaling involves two main pathways: one through myeloid differentiation factor 88 (MyD88), which is used by all TLRs except TLR-3 and is shared by IL-1 and IL-18 receptors, and the other through TRIF, which is solely engaged by TLR-3 and TLR-4 (19). The MyD88 pathway induces early activation of the NF- κ B and activation of the activator protein 1 (AP-1) transcription factors, whereas the TRIF pathway is distinguished by the activation of interferon regulatory factor 3 (IRF-3) and IRF-7, in addition to a late NF- κ B response (20–22).

Previous studies in animal models of acute arthritis have indicated the critical role of MyD88 in SCW-induced joint inflammation (23). Studies on the role of MyD88 during chronic T cell-dependent arthritis and the function of TRIF in any in vivo arthritis model are, however, lacking. Such insight would enable the development of optimal strategies for interfering with pathogenic processes driven by multiple TLRs, while preserving the protective features of TLR response.

In the present study, we extensively investigated the specific functions and the relative contribution of MyD88 and TRIF pathways in different aspects of joint pathology in the mouse model of chronic, predominantly TLR-driven SCW-induced arthritis. We also studied the impact of MyD88 and TRIF signaling on antigen-specific adaptive immune responses, with emphasis on T cell IL-17 production, as key pathogenic events in the disease.

MATERIALS AND METHODS

Induction of chronic SCW arthritis. C57BL/6 mice were purchased from Janvier. MyD88^{-/-} and Trif^{lps2} homozygous mutant (TRIF^{-/-}) mice (24) were kindly provided by Prof. S. Akira (Osaka, Japan) and Prof. B. Beulter (La Jolla, CA), respectively. Mice were housed in filter-top cages, and water and food were provided ad libitum.

Cell wall fragments from *Streptococcus pyogenes* T12 were prepared as described previously (25). Unilateral chronic arthritis was induced by 4 intraarticular injections of 25 μ g of SCW fragments in 6 μ l of phosphate buffered saline (PBS) into the right knee joint on days 0, 7, 14, and 21. In some experiments, the chronic phase was extended by 2 additional injections on days 28 and 35. Animal studies were approved by the Institutional Review Board at Radboud University Nijmegen Medical Centre.

RNA isolation, quantitative polymerase chain reaction (PCR), and oligonucleotide array. Synovial biopsy samples from knee joints were obtained with a 3-mm punch (Stiefel) at the time points indicated. Total RNA isolation and real-time quantitative PCR using SYBR Green Master Mix and an ABI Prism 7000 Sequence Detection system (Applied Biosystems) were performed as described previously (17). Primer sequences (forward and reverse, respectively) were as follows: for GAPDH (housekeeping gene), 5'-GGC-AAA-TTC-AAC-GGC-ACA-3' and 5'-GTT-AGT-GGG-GTC-TCG-CTC-TG-3'; for TLR-3, 5'-CCT-GTT-CTC-TAT-CTG-GGA-AGT-TAA-ACT-G-3' and 5'-TCT-ATC-CCT-CTG-GAA-AGA-CTG-CAT-3'; for TLR-7, 5'-GTG-TGG-TTT-GTC-TGG-TGG-GTT-A-3' and 5'-CCT-GGA-CCT-ACA-CAA-GTC-ACA-TCA-3'; and for TLR-9, 5'-CCT-TCG-TGG-TGT-TCG-ATA-AGG-3' and 5'-GCA-CCC-GCA-GCT-CGT-TAT-A-3'. The C_t value of the gene of interest was corrected to that of GAPDH to obtain the ΔC_t , then the $\Delta\Delta C_t$ was calculated in comparison to synovium obtained on day 0.

For the oligonucleotide array, 1 μ g of total RNA was used for complementary DNA preparation. Generation of biotinylated complementary RNA and subsequent hybridization, washing, and staining of MOE 430_2 oligonucleotide arrays (Affymetrix) were performed according to the *Affymetrix Expression Analysis Technical Manual* for 1-cycle amplification. The arrays were then scanned using a laser scanner (GeneChip Scanner; Affymetrix) and analyzed using Affymetrix GeneChip Operating Software (version 1.4) according to the manufacturer's instructions. Data were further analyzed as described previously (26).

Measurement of joint swelling. Joint swelling was assessed based on the accumulation of ^{99m}Tc in the inflamed joint. Briefly, 0.74 MBq of ^{99m}Tc in 200 μ l of saline was injected subcutaneously, followed by measurement of external gamma radiation in the knee joints. Swelling was calculated as ratio of gamma counts in the right (inflamed) knee joint to gamma counts in the left (control) knee joint. Values >1.1 were considered inflammation.

Histologic assessment. Total knee joints were fixed in 4% formaldehyde for 4 days, then decalcified in 5% formic acid, and embedded in paraffin. Tissue sections (7 μ m) were stained with hematoxylin and eosin to study the inflammatory cell influx and chondrocyte death or with Safranin O to determine proteoglycan depletion, cartilage destruction, and

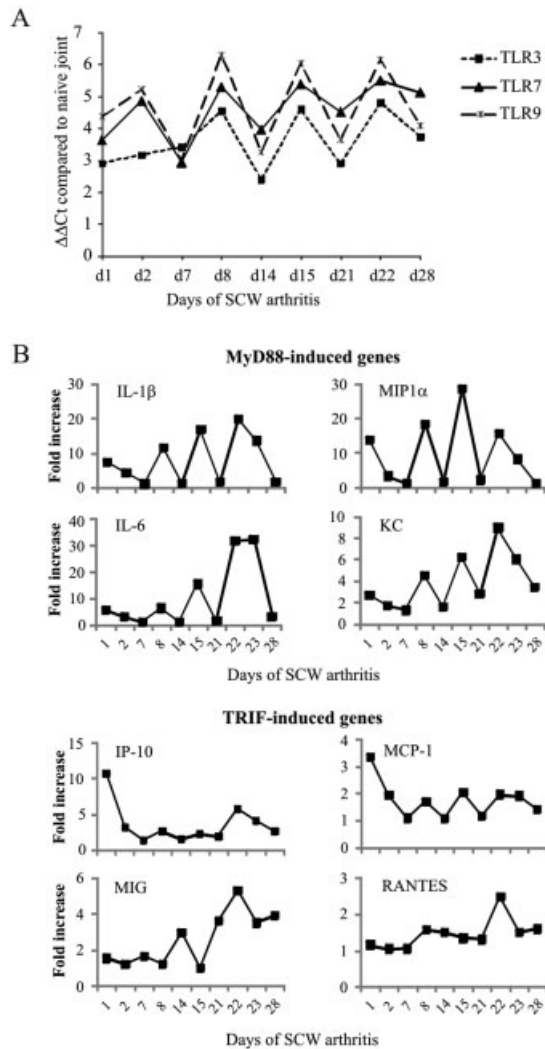


Figure 1. A, Expression of genes for Toll-like receptor (TLR), suggesting both myeloid differentiation factor 88 (MyD88) and TRIF signaling during chronic streptococcal cell wall (SCW)-induced arthritis. Gene expression was measured by quantitative polymerase chain reaction in knee joint synovial tissue at the activation (days 1, 2, 8, 15, and 22) and resolution (days 7, 14, 21, and 28) phases of SCW arthritis. The $\Delta\Delta C_t$ values after correction for GAPDH and for synovium obtained on day 0 (naive) indicate up-regulation of TLR genes during arthritis. B, Synovial expression of representative gene products of MyD88-dependent and TRIF-dependent signaling pathways. Expression of interleukin-1 β (IL-1 β), macrophage inflammatory protein 1 α (MIP-1 α), IL-6, keratinocyte-derived chemokine (KC), which are induced by MyD88, as well as interferon- γ -inducible 10-kd protein (IP-10), monocyte chemoattractant protein 1 (MCP-1), and monokine induced by interferon- γ (MIG), which are induced by TRIF, was measured using an oligonucleotide array during the different phases of arthritis described in A, and the fold change in comparison to synovium obtained on day 0 (naive) was calculated. Values are the mean of 3 mice per group.

bone erosion. Each parameter was scored on a scale of 0–3 by 2 observers (SA-R and BW) in a blinded manner.

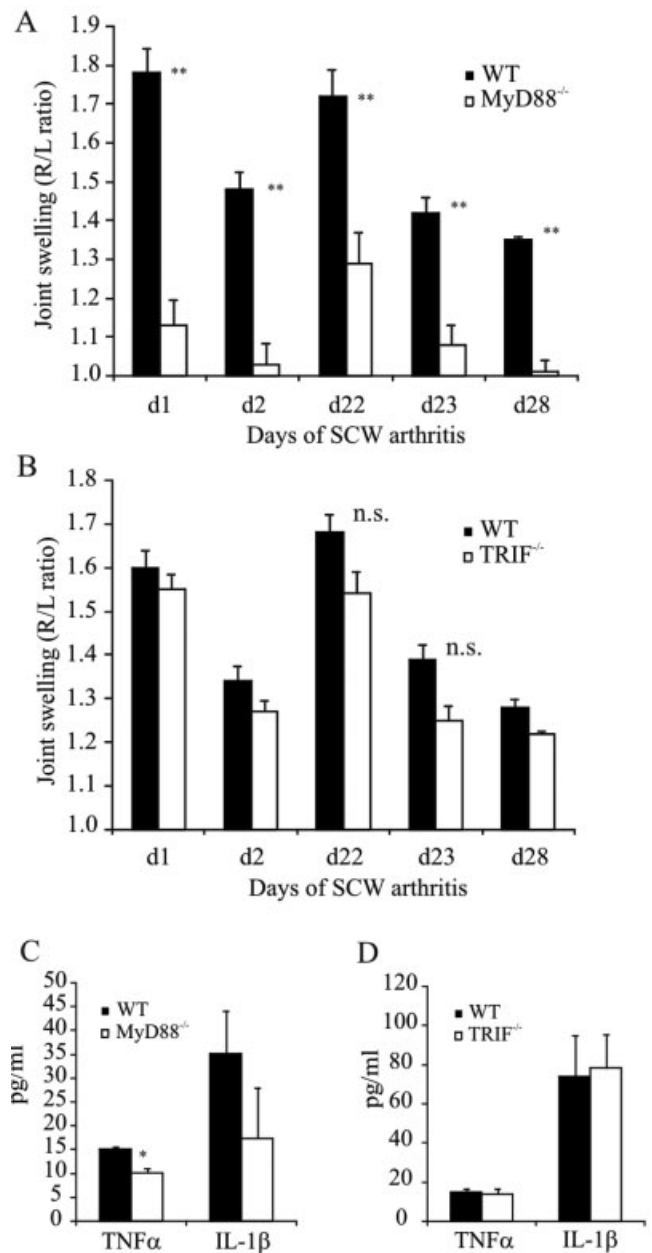


Figure 2. Dependence of streptococcal cell wall (SCW)-induced knee joint swelling and systemic tumor necrosis factor α (TNF α) concentration on myeloid differentiation factor 88 (MyD88) (A and C) and not TRIF (B and D). A and B, Joint swelling on the indicated days of SCW arthritis was determined by measuring ^{99m}Tc uptake in the right (inflamed) knee joint versus the left (control) knee joint during the acute (days 1 and 2) and chronic (days 22, 23, and 28) phases of arthritis in MyD88^{-/-} mice or TRIF^{-/-} mice as compared to wild-type (WT) control mice. C and D, Concentrations of TNF α and interleukin-1 β (IL-1 β) in sera obtained on day 28 from MyD88^{-/-} mice or TRIF^{-/-} mice as compared to wild-type control mice were measured by Bio-Plex cytokine assay. Values are the mean \pm SEM of 6 or more mice per group. * = $P < 0.05$; ** = $P < 0.01$ versus WT mice, by Mann-Whitney U test. NS = not significant.

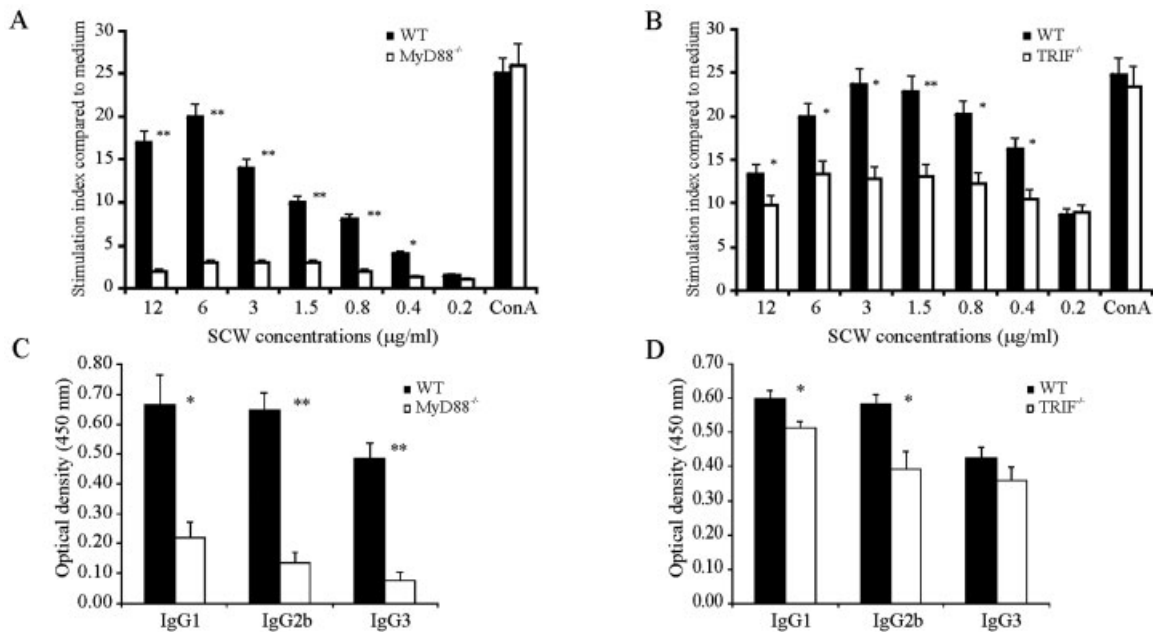


Figure 3. Reduced streptococcal cell wall (SCW)-specific T cell proliferation and antibody production in MyD88^{-/-} mice (A and C) and TRIF^{-/-} mice (B and D) as compared with wild-type (WT) control mice. Nonadherent splenocytes obtained on day 28 of arthritis were stimulated for 72 hours with serial concentrations of SCW fragments or with 1 µg/ml of concanavalin A (Con A), followed by 18 hours of incubation with ³H-thymidine. Anti-SCW antibodies of IgG1, IgG2b, and IgG3 isotype were measured by enzyme-linked immunosorbent assay in sera obtained on day 28. Values are the mean ± SEM of 6 or more mice per group. * = P < 0.05; ** = P < 0.01 versus WT mice, by Mann-Whitney U test.

Lymphocyte stimulation assay. Spleens were isolated on day 28 or day 42 of arthritis (as described below) and then disrupted. Erythrocytes were lysed using 0.16M NH₄Cl (pH 7.2). Cell suspensions were enriched for lymphocytes by allowing the majority of antigen-presenting cells to adhere to plastic culture flasks for 45 minutes. Nonadherent cells (2 × 10⁵/well) containing ~5% dendritic cells were cultured for 72 hours at 37°C in an atmosphere containing 5% CO₂ in RPMI 1640 (Gibco Invitrogen) supplemented with 5% fetal calf serum, 1 mM pyruvate, and 50 mg/liter of gentamicin in the presence of serial concentrations of SCW fragments or 1 µg/ml of concanavalin A (Con A), followed by 18 hours of ³H-thymidine (0.25 µCi/well) incorporation. T cell proliferation was measured using a MicroBeta plate reader (PerkinElmer), and the stimulation index was calculated in comparison to medium. Interferon-γ (IFNγ) and IL-17 production by T cells was assessed in 72-hour culture supernatants using Bio-Plex cytokine assays (Bio-Rad) according to the manufacturer’s instructions.

Measurement of serum cytokines and anti-SCW antibodies. At the end point of the experiments (day 28), serum was collected via the orbital plexus. Cytokine concentrations in the mouse sera were determined using the Bio-Plex cytokine assays (Bio-Rad) following the manufacturer’s instructions. Anti-SCW antibodies in sera were measured using enzyme-linked immunosorbent assay as described previously (17).

Statistical analysis. Group measures are expressed as the mean ± SEM. Statistical significance was assessed using the Mann-Whitney U test performed with GraphPad Prism 4.0 software. P values less than 0.05 were considered significant.

RESULTS

Indication of the activation of both MyD88- and TRIF-dependent pathways in chronic experimental arthritis.

We have previously shown sustained up-regulation of TLR-2 and TLR-4, as well as the adaptor proteins MyD88 and TRIF, in synovial tissue during the acute and chronic phases of SCW arthritis (17). In the present study, we found up-regulation of messenger RNA for TLR-3, TLR-7, and TLR-9 during the course of arthritis. As shown in Figure 1A, expression of these TLRs was enhanced after each SCW injection, and the kinetics followed the arthritis reactivation pattern. Furthermore, in microarray analyses of synovial tissues, we found molecular signatures indicative of MyD88 signaling, including IL-1 receptor-associated kinase 1 (IRAK-1), IL-1β, macrophage inflammatory protein 1α (MIP-1α), IL-6, and keratinocyte-derived chemokine (KC), and those indicative of TRIF signaling, including TRAF-3, IRF-3, IFNγ-inducible 10-kd protein (IP-10), monocyte chemoattractant protein 1 (MCP-1), monokine induced by IFNγ (MIG), and RANTES. Genes with the highest degree of regulation are shown in Figure 1B. The gene expression data collectively suggested that

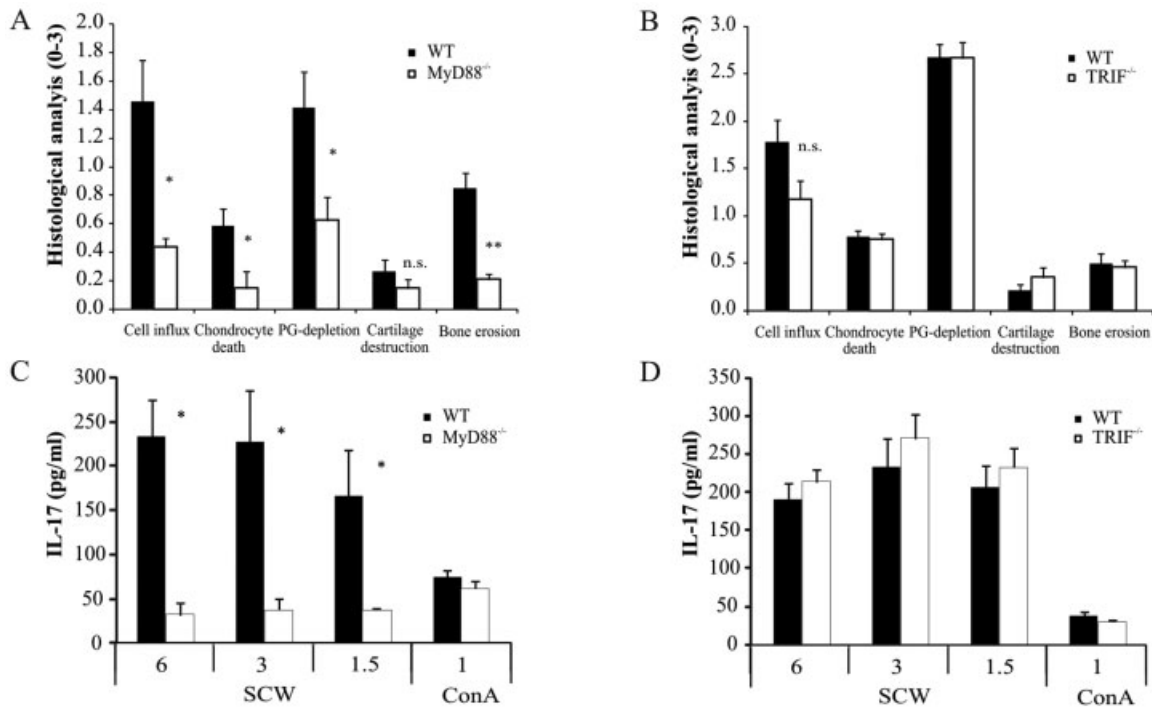


Figure 4. A and B, Dependence of streptococcal cell wall (SCW)-induced histopathologic changes in the knee joint (4 intraarticular injections) on myeloid differentiation factor 88 (MyD88) (A) but not TRIF (B). Knee joints of MyD88^{-/-} mice, TRIF^{-/-} mice, and wild-type (WT) control mice were isolated on day 28 of arthritis. Each histologic feature was scored on a scale of 0–3. PG = proteoglycan. C and D, Reduced systemic production of interleukin-17 (IL-17) in T cells from MyD88^{-/-} mice (C), but not those from TRIF^{-/-} mice (D), with SCW-induced arthritis (day 28). Splenic T cells were stimulated with serial concentrations of SCW fragments or with 1 μ g/ml of concanavalin A (Con A) for 72 hours, and IL-17 levels were measured by Luminex assay. Values are the mean \pm SEM of 6 or more mice per group. * = $P < 0.05$; ** = $P < 0.01$ versus WT mice, by Mann-Whitney U test. NS = not significant.

both MyD88 and TRIF pathways are likely to be activated during chronic experimental arthritis.

Crucial role of MyD88 and redundancy of TRIF in SCW-induced joint swelling. To assess the functional involvement of MyD88 and TRIF signaling pathways in vivo, we induced chronic SCW arthritis in the respective knockout mice. Knee joint swelling during both acute (day 1 and day 2) and chronic (day 22, day 23, and day 28) phases of arthritis was strongly dependent on MyD88 (Figure 2A). In contrast, no significant effects were detected in TRIF^{-/-} mice, despite the tendency toward lower levels of swelling (Figure 2B). Therefore, joint swelling during the entire course of SCW arthritis was mediated through the MyD88, and not the TRIF, pathway.

Reduced levels of proinflammatory cytokines in MyD88^{-/-} mice but not in TRIF^{-/-} mice. We next studied the influence of MyD88 and TRIF signaling pathways on systemic expression of the two relevant proinflammatory cytokines for arthritis, IL-1 β and TNF α . Both

cytokines were detectable in serum during the chronic phase of SCW arthritis (day 28). The concentration of TNF α was significantly lower in sera from mice lacking MyD88, and levels of IL-1 β showed a tendency toward reduction as well (Figure 2C). In contrast, TRIF deficiency had no influence on systemic expression of these two cytokines (Figure 2D). Levels of IFN γ and IL-17 were very low (<2 pg/ml) in sera from mice in all experimental groups.

Contribution of both the MyD88 and the TRIF pathways to antigen-specific T cell proliferation. To assess the SCW-specific T cell proliferation response, splenic T cells were stimulated with various concentrations of SCW. Con A, a pan-T cell mitogen, was used as control. T cell proliferative responses to the nonspecific Con A stimulation were not affected in cells from MyD88^{-/-} mice as well as TRIF^{-/-} mice; however, T cells from MyD88^{-/-} mice and, unexpectedly, to some extent those from TRIF^{-/-} mice showed significantly reduced proliferation upon specific stimulation with the SCW

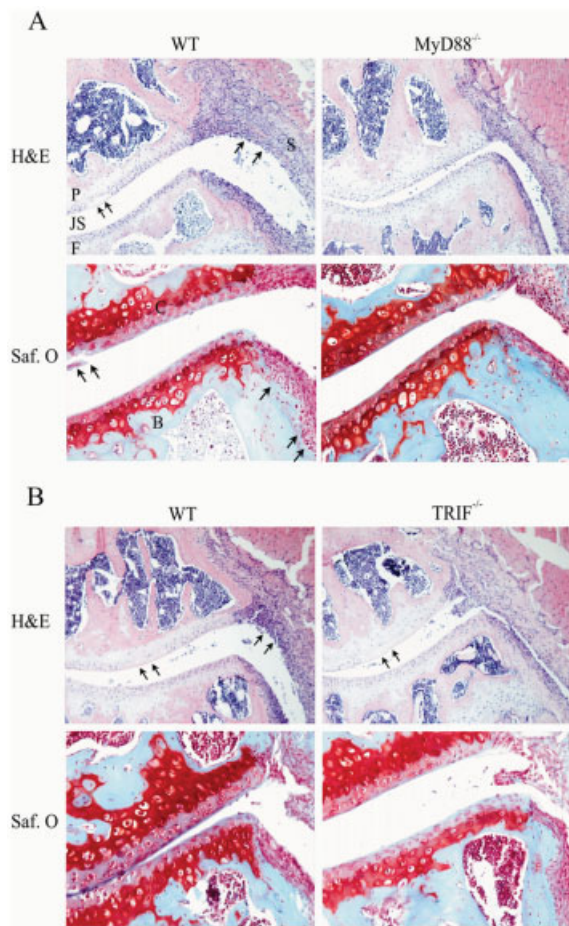


Figure 5. Representative histologic images of the knees of MyD88^{-/-} mice, TRIF^{-/-} mice, and wild-type (WT) control mice, showing myeloid differentiation factor 88 (MyD88) dependency (A) and TRIF redundancy (B) of pathologic changes induced by 4 intraarticular injections of streptococcal cell wall (SCW) fragments. Knee joints were isolated on day 28 and stained with hematoxylin and eosin (H&E) or Safranin O (Saf. O). **Arrows** indicate inflammation and chondrocyte death in H&E-stained sections, and cartilage and bone erosion in Safranin O-stained sections. **P** = patella; **JS** = joint space; **F** = femur; **S** = synovium; **C** = cartilage; **B** = bone. Original magnification × 100 in H&E-stained sections; × 400 in Safranin O-stained sections.

antigen at a broad range of concentrations (Figures 3A and B). Compared to TRIF deficiency, the impact of MyD88 deficiency on antigen-specific T cell responses was much more prominent, as the stimulation index in cells from MyD88^{-/-} mice at all SCW concentrations tested (Figure 3A).

Reduced antigen-specific antibody response in the presence of MyD88 as well as TRIF deficiency. We previously showed that the development of anti-SCW

antibody response arising after the fourth injection of SCW fragments is TLR-4-dependent (17); however, the relative contribution of MyD88 and TRIF pathways remained unknown. Figure 3C shows that MyD88^{-/-} mice have markedly lower circulating levels of anti-SCW antibodies of all isotypes detected. Consistent with the decreased T cell proliferation, TRIF deficiency led to a slight, but significant, reduction in levels of anti-SCW IgG1 and IgG2b (Figure 3D). The IgG3 isotype remained unaffected in TRIF^{-/-} mice. Taken together, MyD88 appeared to be the predominant pathway involved in the anti-SCW antibody response.

Dependence of SCW-induced IL-17 production and chronic joint inflammation and destruction on the MyD88, but not the TRIF, pathway. Next, we extensively examined the involvement of MyD88 and TRIF in the induction of distinct histopathologic hallmarks of chronic arthritis. Histologic assessment of the joints revealed notable protection of MyD88^{-/-} mice against joint inflammation, characterized by synovial hyperplasia and influx of inflammatory cells (Figure 4A). In addition, MyD88^{-/-} mice were clearly protected against chondrocyte cell death and depletion of proteoglycans from articular cartilage, and they also had diminished bone erosion (Figure 4A). In contrast to MyD88, TRIF was dispensable for all pathologic events observed (Figure 4B). Figure 4 also indicates that only low degrees of cartilage destruction were induced during SCW arthritis.

Considering the T cell dependence and the relevance of IL-17 for joint pathology in this model (27,28), we examined T cell cytokine production upon stimulation with the SCW antigen. Consistent with the data from the histologic analysis, the production of IL-17 was substantially reduced by MyD88 deficiency (Figure 4C), but was not affected by TRIF deficiency (Figure 4D), in mice that had received 4 SCW injections. The concentration of IFN γ in SCW-stimulated T cell cultures was very low (<7 pg/ml). This indicates the presence of an antigen-specific Th17-type response under the control of MyD88 signaling.

Representative histologic images of the knee joints are shown in Figure 5. The essential role of MyD88 and the redundancy of TRIF in the chronic pathologic changes in the joint after 4 SCW injections are depicted.

Disrupted TRIF signaling results in enhanced IL-17 production and increased bone erosion during prolonged arthritis. Recent studies indicate the TRIF pathway as a negative regulator of Th17 development during experimental autoimmune encephalomyelitis (29,30). Since the Th17 cells develop late in the course of

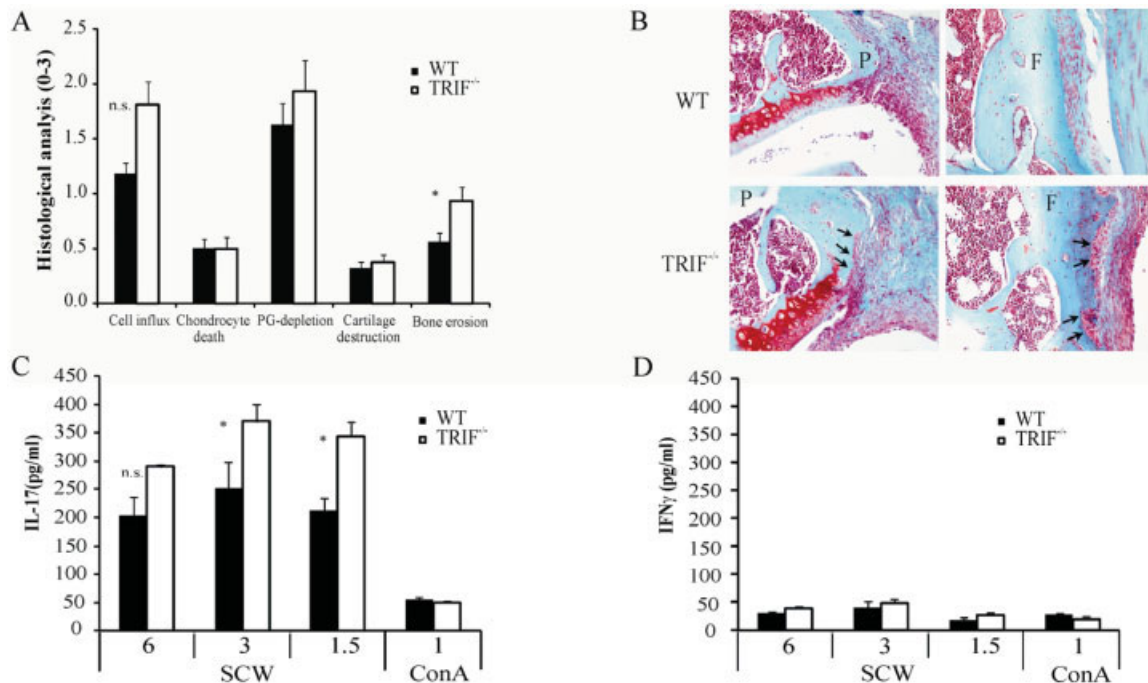


Figure 6. A and B, Increased bone erosion in TRIF^{-/-} mice during the extended phase of streptococcal cell wall (SCW)-induced arthritis. Knee joints of TRIF^{-/-} mice and wild-type (WT) control mice were isolated on day 42 of arthritis after 6 intraarticular injections of SCW fragments. Each histologic feature was scored on a scale of 0–3 (A). PG = proteoglycan. Joint sections were stained with Safranin O (B). Images of the patella (P) are shown on the left and the femur (F) on the right. **Arrows** indicate sites of bone erosion. Original magnification $\times 200$. C and D, Increased production of interleukin-17 (IL-17) (C), but not of interferon- γ (IFN γ) (D), by T cells from TRIF^{-/-} mice with SCW-induced arthritis (day 42). Splenic T cells were stimulated with serial concentrations of SCW fragments or with 1 μ g/ml of concanavalin A (Con A) for 72 hours, and cytokine concentrations were measured by Luminex assay. Values in A, C, and D are the mean \pm SEM of 8 mice per group. * = $P < 0.05$ versus WT mice, by Mann-Whitney U test. NS = not significant.

SCW arthritis (27,28), we extended the chronic phase by administering 2 additional injections of SCW fragments in order to study the impact of the TRIF pathway on Th17-driven prolonged disease. Under these conditions, joint swelling remained unaffected by the lack of TRIF signaling (data not shown). Of great interest, TRIF^{-/-} mice showed a significant increase in bone erosion, which could be observed at both the patellar and femoral sites, whereas no significant effects on cartilage pathology were found (Figures 6A and B). Cell influx tended to be increased by TRIF deficiency as well; however, the difference did not reach statistical significance.

Analysis of the T cell cytokine response revealed that splenic T cells isolated at the extended phase of arthritis produced low levels of IFN γ , but relatively higher amounts of IL-17, upon SCW stimulation (Figures 6C and D). This indicates that the T cell response of Th17 type, rather than Th1 type, was maintained during the prolonged disease. Interestingly, T cells from

TRIF^{-/-} mice with prolonged arthritis produced significantly more IL-17 as compared to those from wild-type mice, when rechallenged with SCW antigen, but not with Con A (Figure 6C). SCW-induced IFN γ production was not influenced by TRIF deficiency (Figure 6D). These findings reveal a negative regulatory function of the TRIF pathway in IL-17-dependent chronic arthritis.

DISCUSSION

Accumulating evidence indicates the involvement of TLRs in arthritis. Therefore, insight into the mechanisms by which TLRs exert their proinflammatory and destructive functions is relevant for the development of efficient therapeutic strategies. In the present study, we studied the specific roles of the two signaling pathways used by TLRs, MyD88 and TRIF, in chronic T cell-driven and IL-17-driven arthritis induced by repetitive exposure to SCW fragments.

Acute SCW-induced arthritis has previously been

demonstrated to be mediated through TLR-2 and, hence, strongly dependent on the MyD88 pathway (23). In contrast, chronic SCW arthritis is independent of TLR-2 and is instead dependent on TLR-4, which controls the production of IL-17 by antigen-specific T cells during the T cell-dependent phase of the disease (17). The present data demonstrate that the MyD88 pathway is critical for the induction of joint swelling and systemic TNF α expression, as well as the antigen-induced adaptive immune responses, during chronic arthritis. An important role of MyD88 in passive (T cell-independent) arthritis induced by the transfer of arthritogenic K/BxN serum has been reported before (31). Here, we provide the first evidence for the involvement of MyD88 in a T cell-driven arthritis model through inducing a Th17 response.

Since MyD88 is also shared by the IL-1 receptor (IL-1R), its role might be related to IL-1R, TLRs, or both. Involvement of IL-1R-induced MyD88 activation in T cell proliferation and antibody production is consistent with a previous report on adaptive responses to a retinal antigen used to induce uveitis in mice (32). Our previous findings on the marked protection of IL-1 α / β ^{-/-} mice from chronic SCW arthritis support this idea as well (27). On the other hand, since IL-1 deficiency only affects T cell proliferation and not the humoral response to SCW (27), whereas MyD88 deficiency affects both (Figures 3A and C), MyD88 activation is unlikely to be restricted to IL-1R and is at least partly initiated by TLRs. A TLR-induced MyD88-mediated pathology is also consistent with the significant role of TLR-4 in the SCW-induced antibody response and IL-17 production (17). Our current knowledge of the involvement of IL-1R, TLRs, and their shared MyD88 signaling indicates that IL-1R-induced MyD88 activation is responsible for T cell proliferation; while TLR-4-induced MyD88 activation is responsible for T cell IL-17 production, possibly through induction of IL-1 β , IL-6, and IL-23, hence creating a Th17-skewing environment (17,27).

In addition to inflammatory cell influx and bone erosion, chondrocyte cell death during chronic SCW arthritis was significantly dependent on MyD88 activation as well. Considering the irreversibility of the latter pathologic phenomenon, targeting MyD88 may be of high therapeutic value in arthritis. A dominant-negative form of MyD88 has been shown to inhibit spontaneous cytokine release from RA synovial membrane cells (33). Nevertheless, a complete blockade of MyD88 signaling in patients would probably increase the risk of noninva-

sive bacterial infections, as has been reported in MyD88-deficient individuals (34).

This is the first study to investigate the role of TRIF in experimental arthritis. Our data provide the first evidence of the involvement of TRIF in antigen-specific adaptive responses to a non-TLR-3-triggering antigen. Previous reports have indicated a role of TRIF in T cell and B cell proliferation induced by TLR-3 triggering (35,36). Stimulation of TLR-3 also induces the differentiation of naive T cells into an IL-21-producing, but not an IL-17-producing, phenotype (37). Furthermore, TRIF signals were found to mediate antibody class switching in B cells toward IgG in response to viral double-stranded RNA (38). In the chronic SCW arthritis model, moderate reductions in T cell proliferation and antibody production in TRIF^{-/-} mice were not sufficient to affect joint pathology. In fact, SCW-directed antibodies seem unlikely to be the main drivers of arthritis, since mice lacking Fc γ receptor types I, II, and III develop the disease to a similar extent as wild-type mice (27). In addition, MyD88 signaling, but not TRIF, is responsible for TLR-4-induced osteoclastogenesis from osteoblasts and is therefore involved in bone resorption (39). These findings may explain the central role of MyD88 and the redundancy of TRIF in TLR-driven arthritis.

TRIF-dependent induction of type I interferons and IL-27 has recently been implicated in the negative regulation of Th17 differentiation and the suppression of autoimmune encephalomyelitis (29,30). Th17 cells are the main producers of IL-17, a potent stimulator of osteoclastogenesis and an inducer of osteoclastic bone resorption either alone or in cooperation with IL-1 β and TNF α (40–42). The highly pathogenic role of IL-17 in inflammatory and destructive processes in RA has now been widely accepted (43,44). The T cell response to the SCW antigen is of the Th17 type rather than the Th1 type, and TLR-4 is an important driver of IL-17 production, which is crucial for the erosive stage of SCW arthritis (Figure 6) (17). In the present study, prolongation of this T cell-dependent erosive stage led to increased bone erosion in the absence of TRIF signaling, an event coincident with increased IL-17 production. These observations suggest a protective role of TRIF signaling on arthritic bone damage and support a possible beneficial effect of TRIF-biased stimulation of TLR signaling under certain circumstances (i.e., Th17-driven disease). A more extensive study of the regulatory role of TRIF in (Th17-driven) arthritis models is still warranted. Furthermore, a TRIF-biased TLR-4 agonist has previously been described (45); however, the impact of its immunostimulatory properties on arthritis remains to

be elucidated. In this context, a possible proinflammatory function of TRIF on, for example, RA fibroblast-like cells, which can be activated by TLR-3 stimulation (46), should be taken into consideration as well.

Taken together, our data indicate a central role of MyD88 signaling in chronic T cell-mediated IL-17-driven arthritis. TRIF, in contrast, is redundant for joint pathology and may even down-modulate bone erosion via inhibition of IL-17 production. We postulate that under conditions of disease, such as RA, in which Th17 cells are considered pathogenic, interference with TLR activation should preferably target only MyD88 and leave the TRIF pathway intact.

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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.

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ROLE OF THE STUDY SPONSOR

Novartis AG performed the microarray studies but otherwise had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by Novartis AG.

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