RNA Released From Necrotic Synovial Fluid Cells Activates Rheumatoid Arthritis Synovial Fibroblasts Via Toll-like Receptor 3

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Objective. To assess the expression of Toll-like receptor 3 (TLR-3) protein in synovial tissues and cultured synovial fibroblasts obtained from patients with rheumatoid arthritis (RA) and osteoarthritis (OA) and to investigate the consequences of stimulation of cultured synovial fibroblasts with TLR-3 ligands.

Methods. TLR-3 expression in synovial tissues was determined by immunohistochemistry and immunofluorescence, and expression in cultured RA synovial fibroblasts (RASFs) was determined by fluorescence-activated cell sorting and real-time polymerase chain reaction techniques. TLR-3 signaling was assessed by incubating RASFs with poly(I-C), lipopolysaccharide, palmitoyl-3-cysteine-serine-lysine-4, or necrotic synovial fluid cells from RA patients in the presence or absence of hydroxychloroquine or Benzonase. Subsequent determination of interferon-β (IFNβ), CXCL10, CCL5, and interleukin-6 (IL-6) protein production in the culture supernatants was performed by enzyme-linked immunosorbent assays.

Results. TLR-3 protein expression was found to be higher in RA synovial tissues than in OA synovial tissues. TLR-3 expression was localized predominantly in the synovial lining, with a majority of the TLR-3–expressing cells coexpressing fibroblast markers. Stimulation of cultured RASFs with the TLR-3 ligand poly(I-C) resulted in the production of high levels of IFNβ, CXCL10, CCL5, and IL-6 protein. Similarly, coincubation of RASFs with necrotic synovial fluid cells from patients with RA resulted in up-regulation of these cytokines and chemokines in a TLR-3–dependent manner.

Conclusion. Our findings demonstrate the expression of TLR-3 in RA synovial tissue and the activation of RASFs in vitro by the TLR-3 ligand poly(I-C) as well as by necrotic RA synovial fluid cells, and indicate that RNA released from necrotic cells might act as an endogenous TLR-3 ligand for the stimulation of proinflammatory gene expression in RASFs.

Recent evidence indicates that the innate immune system plays a decisive role in host defense and self-tolerance (1). Cells of the innate immune system express pattern-recognition receptors, such as the Toll-like receptors (TLRs), which sense certain highly conserved structures that are found on many different bacterial and viral products. The recognition of specific microbial structures, such as lipopolysaccharide (LPS), by TLRs results in the up-regulation of costimulatory molecules in antigen-presenting cells, providing the second signal necessary for generating efficient T cell responses against invading pathogens (2). In contrast, in the absence of a costimulatory TLR signal, T cell receptor stimulation will be followed by a silencing of the T cells. Hence, the innate immune system controls subsequent adaptive immune responses.

Because of this important regulatory role of TLRs, it has been speculated that aberrant TLR signaling may be involved in the generation of autoimmunity. Several animal models of arthritis have been shown to be at least partly dependent on signaling via TLRs (3,4). Moreover, it has been shown that the injection of bacterial products, such as the TLR-2 ligand peptidoglycan or the TLR-9 ligand CpG DNA, into the joints of mice results in arthritis.

Recently, we demonstrated the expression of TLR-2 in rheumatoid arthritis (RA) synovial tissues (5).
We also found that activation of synovial fibroblasts in culture with TLR-2 ligands results in the up-regulation of TLR-2 expression and the production of proinflammatory cytokines (6). Moreover, a variety of chemokines typically found in the synovial fluid of RA patients were found to be secreted by fibroblasts stimulated via TLR-2 (7). While certain TLRs are expressed in joint tissue, it is less clear whether specific TLR ligands are present in the joints of patients with nonseptic arthritis. Peptidoglycans and bacterial DNA derived from gut-colonizing bacteria have been detected in the joints of patients with RA but have also been found in the joints of patients with osteoarthritis (OA) (8). The pathogenetic relevance of these bacterial products, however, remains to be established. More interest has been raised by the demonstration that endogenous ligands, some of which can be found in joints, are able to specifically activate certain TLRs. Recently, activation of TLR-3 by double-stranded RNA (dsRNA) released from necrotic cell lines has been described (9). Injection of dsRNA into mice resulted in a self-limited arthritis, suggesting that TLR-3 signaling may contribute to the pathogenesis of arthritis (10). However, it is not clear whether synovial cells express functional TLR-3.

In the present study, we investigated the possible role of TLR-3 in RA by analyzing TLR-3 expression in synovial tissues and isolated cultured synovial fibroblasts. Our results indicate higher TLR-3 protein expression in RA synovial tissues than in OA synovial tissues. Moreover, we found that RNA released by necrotic synovial fluid cells derived from patients with RA can act as an endogenous ligand for TLR-3 on cultured RA synovial fibroblasts (RASFs). The resulting TLR-3 activation induced the expression of type I IFN as well as the expression of Th1-associated chemokines, such as CXCL10 and CCL5. These results suggest an important role of TLR-3 in the activation of synovial fibroblasts in RA.

MATERIALS AND METHODS

Patients and tissue preparation. Synovial tissues were obtained from patients with RA and OA who were undergoing synovectomy or joint replacement surgery (Department of Orthopedic Surgery, Schulthess Clinic, Zurich, Switzerland). Synovial tissues were divided into 3 parts and were used to isolate synovial fibroblasts for cell culture, to obtain synovial tissues for immunohistochemistry, and to extract total RNA. All RA patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the classification of RA (11).

Immunohistochemistry and immunofluorescence analyses. Immediately after surgery, synovial tissues were embedded in OCT compound (TissueTek TT 4583; Sakura Finetech, Torrance, CA) and snap-frozen in liquid nitrogen. Embedded synovial tissues were maintained at −80°C until cryosectioned. Seven-micrometer sections were prepared, fixed in acetone, dried, and then rehydrated in phosphate buffered saline (PBS).

Endogenous peroxidase activity was blocked using 0.1% H2O2, and endogenous biotin was blocked using a biotin blocking kit from Vector (Burlingame, CA). To inhibit non-specific binding, slides were incubated for 1 hour in blocking solution (2% fetal calf serum [FCS] in Tris buffered saline [TBS], pH 7.4). Slides were then incubated for 1 hour with 5 μg/ml of affinity-purified goat anti-human TLR-3 polyclonal antibody (MBL International, Woburn, MA).

After the primary antibody reaction, the sections were incubated for 30 minutes with biotinylated rabbit anti-goat IgG (DakoCytomation, Glostrup, Denmark) in TBS with 1% bovine serum albumin, followed by incubation for 30 minutes with horseradish peroxidase (HRP)–conjugated streptavidin complex (Vectastain Elite ABC kit; Vector). HRP-labeled cells were visualized using aminoethylcarbazole chromogen substrate (DakoCytomation). Nuclei were counterstained with hematoxylin.

To identify macrophages, slides were additionally incubated for 1 hour with 3 μg/ml of monoclonal mouse anti-human CD68 (DakoCytomation). To detect fibroblasts, slides were additionally incubated with 3 μg/ml of monoclonal mouse anti-human vimentin (DakoCytomation). Bound mouse primary antibodies were detected using alkaline phosphatase (AP)–conjugated rabbit anti-mouse IgG antibody (DakoCytomation). AP-labeled cells were visualized using Fast Blue BB reagent: naphthol-AS-MX phosphate dissolved in N,N-dimethylformamide was mixed immediately before use with Fast Blue BB dissolved in TBS, pH 8.5 (Sigma, Basel, Switzerland) and levamisole solution (DakoCytomation). In control experiments, goat IgG and matched mouse IgG isotype controls were used instead of the primary antibodies.

For immunofluorescent double staining, we used 10 μg/ml of fluorescein isothiocyanate–conjugated monoclonal mouse antifibroblast antibody ASO2 (Dianova, Hamburg, Germany). TLR-3 protein was detected using the same primary antibodies as for the immunohistochemical analysis, followed by incubation with 3 μg/ml of phycoerythrin (PE)–conjugated donkey anti-goat antibodies (Jackson ImmunoResearch, Soham, UK). All steps were performed at room temperature.

Isolation and culture of synovial fibroblasts. Immediately after surgery, the synovial tissue was minced and digested with Dispase at 37°C for 60 minutes. After washing, the cells were grown in Dulbecco's minimum essential medium (Gibco Invitrogen, Basel, Switzerland) supplemented with 10% FCS, 50 IU/ml penicillin/streptomycin, 2 mM L-glutamine, 10 mM HEPES, and 0.2% Fungizone (all from Gibco Invitrogen). Cell cultures were maintained at 37°C in a humidified incubator in an atmosphere of 5% CO2. For the experiments, cultured synovial fibroblasts were used between passages 4 and 8.

Reagents and stimulation assays. Cultured synovial fibroblasts were grown in 12-well culture plates (6 × 104 RASFs/well) and subsequently stimulated with the following agents: poly(I:C) (20 μg/ml; InvivoGen, San Diego, CA), LPS from Escherichia coli (100 ng/ml; List Biological Laboratories, Campbell, CA), palmitoyl-3-cysteine-serine-lysine-4 (Pam3CSK4)
TLR-3 with the macrophage marker CD68 was also identified; however, most of the cells expressing TLR-3 did not express CD68 (Figure 1H).

To verify the differential expression of TLR-3 in RA compared with OA synovial tissues, real-time PCR of total RNA extracts from synovial tissue was performed. TLR-3 mRNA could be detected in all 7 RA samples and all 4 OA samples tested. However, expression of TLR-3 mRNA was significantly higher in the RA synovial tissue (6-fold difference; mean ± SEM ΔCt...
Expression of TLR-3 protein by synovial fibroblasts in vitro. To confirm the results of the immunohistochemical analysis, basal TLR-3 protein expression of cultured synovial fibroblasts was assessed by flow cytometry. A majority of RASFs expressed TLR-3 protein constitutively (mean ± SEM mean fluorescence intensity [MFI] 82.1 ± 8.8; isotype control MFI 32.9 ± 4.9). Moreover, stimulation of RASFs with the TLR-3 agonist poly(I-C) up-regulated the expression of TLR-3 protein by almost 2-fold (MFI 147.0 ± 34.8) as compared with unstimulated cultures (Figure 2A).

To assess the specificity of TLR-3 up-regulation, RASFs and OASFs were stimulated with the TLR-3 ligand poly(I-C), with the TLR-4 ligand LPS, or with the TLR-2 ligand Pam₃CSK₄ (Figure 2B). TLR-3 mRNA expression was analyzed by real-time PCR 24 hours following stimulation. Poly(I-C) up-regulated TLR-3 mRNA in RASFs a mean ± SEM of 14.2 ± 2.0-fold and in OASFs a mean ± SEM of 9.3 ± 2.4-fold compared with unstimulated cultures, with a statistically significant difference between RASFs and OASFs. LPS stimulation induced a small up-regulation of TLR-3 mRNA (3.0 ± 1.0-fold in RASFs and 2.2 ± 0.8-fold in OASFs), but the difference was not statistically significant. Pam₃CSK₄ did not show any TLR-3 mRNA up-regulation in either case. These results demonstrate the constitutive expression of TLR-3 in synovial fibroblasts and a preferential up-regulation of TLR-3 in synovial fibroblasts from patients with RA in the presence of the TLR-3 agonist poly(I-C).

Induction of IFNβ, CXCL10, CCL5, and IL-6 in RASFs following stimulation with poly(I-C). In contrast to TLR-2 and other known TLR family members, TLR-3 signals exclusively through a myeloid differentiation factor 88 (MyD88)–independent pathway using the adaptor molecule TRIF, whereas TLR-4 uses both the MyD88-dependent and the MyD88-independent pathways, with TRIF and TRAM as adaptors. In addition to NF-κB and MAP kinases, the TRIF-dependent pathways activate the transcription factor interferon regulatory factor 3 (IRF-3), leading to the induction of type I IFN expression. To assess the TRIF-dependent pathways in cultured RASFs, cells were stimulated with poly(I-C), LPS, and Pam₃CSK₄. After 24 hours of stimulation, the supernatants were tested by ELISA for the presence of TRIF-dependent and TRIF-independent cytokines and chemokines.

IFNβ in RASFs was highly induced by poly(I-C) and was induced to a lesser extent by LPS, whereas Pam₃CSK₄ had no effect (Figure 3). Moreover, the Th1-associated chemokines CXCL10 and CCL5 were most strongly induced after stimulation with poly(I-C). In response to LPS, RASFs produced significant amounts of CXCL10 and CCL5 protein, whereas Pam₃CSK₄ induced only CCL5. High amounts of the proinflammatory cytokine IL-6 were detected in supernatants from RASF cultures stimulated with each of the 3 TLR ligands; however, poly(I-C) was the most effective stimulator (Figure 3).
Induction of CXCL10 expression by IFNβ released by poly(I-C) stimulated RASFs. Recent studies suggest that type I IFNs are able to induce CXCL10 gene expression (12). We therefore analyzed whether IFNβ regulates the chemokine gene expression in poly(I-C)–stimulated RASFs. RASF cultures were stimulated with the indicated Toll-like receptor ligands or were left untreated. Concentrations of IFNβ, CXCL10, CCL5, and IL-6 in the culture supernatants were determined after 24 hours with enzyme-linked immunosorbent assays. Values are the mean and SEM of 4–6 different RASF cultures. * = P < 0.02 versus untreated cultures. LPS = lipopolysaccharide; Pam3CSK4 = palmitoyl-3-cysteine-serine-lysine-4.

We therefore analyzed whether exogenous IFNβ was able to stimulate CXCL10 production in RASFs. RASFs were incubated with various concentrations of IFNβ for 24 hours, and CXCL10 protein production was subsequently analyzed by ELISA. Consistent with the findings shown in Figure 4A, exogenous IFNβ induced CXCL10 protein secretion in a dose-dependent manner (Figure 4B).

Inhibition of TLR-3 signaling in synovial fibroblasts by HCQ. HCQ inhibits endosomal acidification, on which signaling of the intracellularly located TLRs, such as TLRs 3, 7, 8, and 9, depend. Since synovial fibroblasts do not express TLRs 7, 8, and 9, we used HCQ as a specific TLR-3 inhibitor in cultures of RASFs stimulated with poly(I-C), LPS, or Pam3CSK4. The induction of IFNβ, CXCL10, CCL5, and IL-6 in response to poly(I-C) was almost completely abolished when RASFs were pretreated with 2 μg/ml of HCQ (Table 1). In contrast, HCQ did not have such an effect.
on the cytokine and chemokine production by RASFs in response to LPS or Pam3CSK4.

Necrotic synovial fluid cell stimulation of RASFs via TLR-3. It has been shown that mRNA released from necrotic cells can act as an endogenous ligand for TLR-3 in dendritic cells (9). Since necrotic cells can be detected in the synovial fluid of patients with RA, we examined the stimulatory effects of necrotic synovial fluid cells from RA patients on cultured RASFs. RASFs cultured in the presence of necrotic synovial fluid cells up-regulated the expression of IFNβ mRNA and the production of IL-6, CXCL10, and CCL5 protein (Figure 5A). The stimulatory effect was dependent on the concentration of necrotic cells added to the cultures (Figure 5B). The addition of HCQ resulted in a significant reduction of the stimulatory effect of necrotic synovial fluid cells, suggesting TLR-3 dependency.

To examine whether RNA is the effector molecule for TLR-3 activation, necrotic synovial fluid cells were incubated with Benzonase for 12 hours at 4°C prior to stimulation. Benzonase is an endonuclease that degrades all forms of RNA into oligomers of 2–5 nucleotides in length. RNA digestion from necrotic synovial fluid cells by Benzonase significantly decreased the expression of IFNβ mRNA as well as the protein concentrations of the tested chemokines and cytokines in the RASF cultures (Figure 5A). These results suggest that RNA derived from necrotic synovial fluid cells stimulates synovial fibroblasts via TLR-3.

**DISCUSSION**

Activation of the innate immune system via Toll-like receptors leads to the induction of the expression of proinflammatory cytokines and chemokines. Both play an important role in the development of the joint inflammation characteristic of RA. We have previously demonstrated the expression of functional TLR-2 in synovial fibroblasts from patients with RA (5,6). The finding that TLR-2 expression was increased in RA synovial tissue as compared with OA synovial tissue suggested that TLR signaling is active in RA. Further evidence of a role of TLRs in arthritis is provided by studies of animal models of arthritis. Streptococcal cell wall–induced arthritis was shown to be dependent on TLR-2 signaling (4). Moreover, it was shown that LPS can circumvent the need for IL-1 for development of antibody-transfer arthritis in the K/BxN model, demonstrating a role of TLR ligands in later stages of the pathogenesis of arthritis (3). Although microbial products such as bacterial DNA and peptidoglycans have been detected in the joints of patients with RA, their pathogenic significance is unclear.

The recent identification of endogenous ligands for several different TLRs has generated great interest because of their potential importance for autoimmunity. For example, heat-shock proteins 60 and 70 have been shown to be ligands of TLR-2 and TLR-4 and have previously been implicated in the pathogenesis of RA (13–16). Fibrinogen is another TLR-4 ligand that is present in joints (17). Moreover, it has been demonstrated that chromatin-containing immune complexes may give rise to the production of rheumatoid factor autoantibodies by the synergistic engagement of B cell receptor and TLR-9 (18). Necrotic cells have been demonstrated to activate macrophages in a TLR-2–dependent manner, although the nature of the ligand remains obscure (19). Viral dsRNA is a ligand for TLR-3, whereas the single-stranded form activates TLR-8 (20,21). Kariko et al (9) have demonstrated that stimulation of TLR-3 is not restricted to viral RNA, but also results from incubation with in vitro–transcribed mRNA and mRNA released from necrotic cells. These

### Table 1. Specific inhibition of TLR-3 in RASFs by treatment with HCQ*

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<th></th>
<th>% IFNβ</th>
<th>% CXCL10</th>
<th>% CCL5</th>
<th>% IL-6</th>
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<tr>
<td>Poly(I-C)</td>
<td>100</td>
<td>100</td>
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<td>Poly(I-C) + HCQ</td>
<td>5.0 ± 2.8</td>
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<td>LPS</td>
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<tr>
<td>LPS + HCQ</td>
<td>109.2 ± 45.5</td>
<td>101.2 ± 22.7</td>
<td>102.7 ± 23.1</td>
<td>110.5 ± 17.4</td>
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<td>ND</td>
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<tr>
<td>Pam3CSK4 + HCQ</td>
<td>ND</td>
<td>ND</td>
<td>91.4 ± 5.3</td>
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* Rheumatoid arthritis synovial fibroblasts (RASFs) were stimulated for 24 hours with the indicated Toll-like receptor (TLR) ligands in the presence or absence of 2 μg/ml of hydroxychloroquine (HCQ). Culture supernatants were collected, and protein levels were determined by enzyme-linked immunosorbent assay. Values are the mean ± SEM of at least 3 individual experiments. IFNβ = interferon-β; IL-6 = interleukin-6; LPS = lipopolysaccharide; Pam3CSK4 = palmitoyl-3-cysteine-serine-lysine-4; ND = not done.
results have established that dsRNA sequences contained in mRNA may serve as an endogenous TLR-3 ligand.

In this study, we analyzed TLR-3 expression in synovial tissues and its functional aspects in vitro. TLR-3 was found to be expressed in all samples derived from patients with RA and OA; however, staining of OA sections was weak compared with the staining of RA sections. Stimulation of cultured RASFs with poly(I-C) resulted in increased TLR-3 expression. These findings are compatible with active TLR-3 signaling in RA, although it remains unclear whether increased TLR expression represents a secondary phenomenon related to the inflammatory reaction in the joints. We therefore analyzed the consequences of TLR-3 stimulation in synovial fibroblasts in vitro.

Stimulation with the TLR-3 ligand poly(I-C) resulted in the up-regulation of the proinflammatory cytokine IL-6 and the chemokine CCL5. This effect of poly(I-C) is similar to the expression profile seen with TLR-2 stimulation (6) and is consistent with the activation of NF-κB, which has been shown to occur upon TLR-3 stimulation in a MyD88-independent manner via tumor necrosis factor receptor–associated factor 6 (22).

Contrary to all other TLRs (except TLR-4), TLR-3 signals via a MyD88-independent pathway using the adapter molecule TRIF, which leads not only to NF-κB activation, but also to the activation of IRF-3, inducing the expression of type I IFN and IFN-responsive genes. Correspondingly, poly(I-C) stimulation of RASFs induced the expression of IFN-β and the chemokine CXCL10. The up-regulation of CXCL10 by poly(I-C) seen in RASFs was at least partly dependent on IFN-β production, since it could be inhibited by anti-IFN-β antibodies. The specificity of the effect of poly(I-C) was confirmed by using HCQ. HCQ blocks endosomal acidification and thereby inhibits signaling of intracellularly located TLRs, such as TLRs 3, 7, 8 and 9. Since TLRs 7, 8, and 9 are not expressed in RASFs (ref. 6 and Brentano F, et al: unpublished observations), HCQ acts as a specific TLR-3 blocker.

Both IFN-β and CXCL10 have previously been described as being up-regulated in RA (23–26). CXCL10 is a chemokine that attracts activated T cells and natural killer cells. Expression of CXCR3, the receptor for CXCL10, has been associated with a Th1 phenotype (27). Whereas IFN-γ is the main inducer for CXCL10 expression in peripheral blood mononuclear cells, dsRNA has a stronger inducing effect in fibroblasts, acting synergistically with IFN-γ (28). In addition, up-regulation of CXCL10 secretion was demonstrated in cocultures of fibroblast-like synoviocytes and leukocytes, and was dependent on cell contact but independent of
there was no indication of an inhibitory effect of IFN receptor antagonist (34,35). In our experiments, however, survival, another study demonstrated induction of IL-1 cell blasts (26), possibly resulting in inappropriate T cell IFN-tressed clinical trial, however, reported no benefit of daily development of arthritis (31,32). A recent randomized con-primates with collagen-induced arthritis inhibited the de-
eration of this cytokine. IFN treatment in mice as well as primatest with collagen-induced arthritis inhibited the de-
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trolled clinical trial, however, reported no benefit of daily administration of IFNβ (33). In vitro studies suggest ben-
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Type I IFNs have pleiotropic effects on the cells of the immune system. They induce activation of immu-
ture dendritic cells, promote B and T cell maturation, keep activated CD8+ and CD4+ cells alive, and promote macrophage maturation and inducible nitric oxide synthase production. In contrast, they also have antiproliferative and proapoptotic effects on T cells (for review, see ref. 30). With regard to IFNβ, available data from mouse models of arthritis suggest a beneficial effect of continuous admin-
istration of this cytokine. IFNβ treatment in mice as well as primates with collagen-induced arthritis inhibited the de-
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TLR pathways are activated during infections, contributing to an efficient host defense, but are self limited with the clearing of the microorganism. Uncon-
trolled TLR signaling could theoretically lead to auto-
immune disease; however, so far, no specific disease has been linked to a defect in TLR regulation. In addition to up-regulated TLR expression, the availability of TLR ligands may be responsible for the enhanced activity of the signaling pathways.

Necrotic cells may be found in RA joints as a result of inflammatory and destructive processes. Whereas necrotic cells can easily be detected in synovial fluid (e.g., with propidium iodide staining, we consistently found at least 10% necrotic cells in synovial fluid from RA patients), necrotic cells are usually not present in large amounts in the synovial tissue. However, apoptotic cells in synovial tissue may undergo secondary necrosis (36).

In an attempt to assess the effects of necrotic cells on synovial fibroblasts under conditions as close as possible to those found in vivo in the arthritic joint, we used necrotic synovial fluid cells from RA patients to stimulate cultured RASFs. Interestingly, the necrotic synovial fluid cells efficiently up-regulated proinflammatory cytokines and chemokines, with a profile resembling that of RASFs stimulated with poly(I-C). The induction of IL-6, CCL5, and CXCL10 protein and IFNβ mRNA was significantly reduced by the addition of HCQ, which inhibited TLR-3 signaling, but not TLR-2 and TLR-4 signaling. Similarly, the RNA-degrading enzyme Benzo-
nase significantly decreased the response of RASFs to the necrotic synovial fluid cells. These results indicate that mRNA containing short dsRNA sequences that are released from necrotic synovial fluid cells are sufficient to activate cultured human RASFs, resulting in the induc-
tion of proinflammatory gene expression. These findings extend those of a recent study that demonstrated the activation of human endometrial cells with U1 RNA containing dsRNA repeats (37). The U1 RNA was derived from U1 RNP, autoantibodies to which can be found in a subset of patients with collagen vascular disease.

Cell-free synovial fluid from patients with RA also stimulated RASFs. However, in this case, the presence of cytokines such as tumor necrosis factor α, IL-1β, or IFNβ may be responsible for the stimulatory effect. This is suggested by the fact that the activation induced by the cell-free synovial fluid could not be blocked by HCQ (data not shown).

In summary, we have demonstrated the expression of TLR-3 in a majority of synovial fibroblasts from the joints of patients with RA and the activation of RASFs in vitro by dsRNA of synthetic or endogenous origin. Based on our findings, we propose that, in addition to stimulatory effects of cytokines, local tissue-destruc
tive events, whether caused by an infection involving the joint or by noninfectious processes, may lead to the release of endogenous dsRNA, activating tissue-
resident synovial fibroblasts via TLR-3. In susceptible individuals, increased TLR-3 expression and expression of tissue-destructive enzymes by RASFs might then contribute to the perpetuation of the disease.

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REFERENCES

ACTIVATION OF RASFs BY NECROTIC SYNOVIAL FLUID CELLS


