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Role of Interferon Regulatory Factor 7 in Serum Transfer Arthritis: Regulation of Interferon β production

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Abstract

Objective—Innate immune responses activate synoviocytes and recruit inflammatory cells into the rheumatoid joint. Type I interferons (IFN) play a role in autoimmunity and IFN gene transcription is activated by IFN-regulatory factors (IRF) in response to innate sensor recognition. This study examined the effect of genetic deficiency of IRF7 in a passive K/BxN serum transfer model of arthritis.

Methods—Passive transfer arthritis was induced in *Irf7*^{-/-} mice and additional groups were treated with IFN β or poly (I-C). Clinical arthritis scores, histology, microcomputed tomography, and synovial tissue Q-PCR were performed. Mouse serum was analyzed by ELISA.

Results—In the K/BxN passive model, arthritis severity was significantly increased in *Irf7*^{-/-} mice compared with wild type (WT). In addition, synovial and serum IFN β expression was decreased, potentially contributing to increased arthritis. *Irf7*^{-/-} mice injected with replacement IFN β had a decrease in arthritis. Poly (I-C) treatment diminished arthritis in *Irf7*^{-/-} mice, restored synovial IFN β gene expression, and increased serum IFN β . *In vitro* studies demonstrated that poly (I-C) stimulation of *Irf7*^{-/-} fibroblast-like synoviocytes (FLS) resulted in increased induction of pro-inflammatory gene expression compared with WT FLS; however, IFN β expression was not significantly different. In contrast, *Irf7*^{-/-} macrophages showed significantly less induction of IFN β in response to poly (I-C) stimulation.

Conclusions—IRF7 deficiency exacerbated arthritis and replacement treatment with IFN β or poly (I-C) decreased arthritis severity. Both macrophage and synoviocyte specific roles for IRF7 likely contribute to increased arthritis. IRF7 might play an anti-inflammatory role in passive transfer arthritis through regulation of macrophage IFN β production.

Keywords

transcription factors; signal transduction; inflammation; rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is characterized by critical alterations in synovial inflammation, matrix degradation, and cellular proliferation. This destructive process is controlled by a number of intracellular signaling pathways that regulate gene expression in the rheumatoid synovial intimal lining (1, 2). Innate sensors induce secretion of type I interferons (IFN α and IFN β) that serve as key sentinel cytokines and play a complex role in autoimmunity (3). Type I IFN can induce expression of hundreds of IFN-stimulated genes that can both

promote immunity and other functions that suppress inflammation and immune responses. The IFN-regulatory factors (IRF) are involved in innate receptor signaling and are mainly responsible for induction of the type I IFN response downstream of pattern recognition receptors (4).

Type I IFN can promote immunity but can also suppress immunity and inflammation. IFN β inhibits inflammatory arthritis in mouse models. Mice with collagen-induced arthritis injected with fibroblasts expressing IFN β have decreased arthritis severity and bone and cartilage destruction (5). IFN β also decreases matrix metalloproteinases (MMP), IL-1, and TNF production by fibroblast-like synoviocytes (FLS) (6). However, a clinical trial using IFN β in patients with RA showed minimal efficacy despite decreased synovial IL-1, IL-6, and MMP1 (7). Similar to the IFN response, innate receptor activation can also have multiple, diverse effects depending on dose and administration of the ligand. For example, TLR activation has been shown to contribute to inflammation in autoimmune diseases; however, repeated exposure to a TLR agonist such as polyinosinic polycytidylic acid [poly (I-C)] induces a hypo-responsiveness to further TLR stimulation (8, 9). In addition, poly (I-C) has been previously studied in the K/BxN passive antibody-mediated arthritis model and resulted in a decrease in arthritis that was dependent on IFN α/β receptor signaling (9, 10). Of interest, IKK ϵ , an IKK-related kinase that activates IRF3 and IRF7 in response to dsRNA innate receptor ligation, was studied in murine passive K/BxN transfer arthritis (10). Low dose IFN β treatment enhanced the anti-inflammatory effect of IKK ϵ deficiency possibly by increasing production of IL-1 receptor antagonist (IL-1RA).

IRF3 and IRF7 are key regulators of type I IFN gene expression (11). We previously demonstrated that IRF3 is expressed and activated in rheumatoid synovium, suggesting the potential importance of IRF3 in RA (12). In vitro siRNA knockdown indicated that IRF3, but not IRF7, played a key role in human FLS type I IFN responses as well as traditionally AP-1 regulated gene expression (13). In mouse embryonic fibroblasts and plasmacytoid dendritic cells, IRF7 is considered the master regulator of type I IFN immune responses (14). Constitutive and ubiquitous IRF3 mainly controls initial IFN β production, whereas basal expression of IRF7 is low in most cell populations, but induced by type I IFN. Nuclear translocation of IRF7 can induce IFN β gene expression and also numerous subtypes of IFN α . We hypothesized that IRF7 regulates synovial inflammation and contributes to innate immunity and the clinical course of arthritis, possibly by altering IFN β and other IFN-regulated genes in a cell specific manner. Further understanding of this regulatory mechanism will have major implications for targeting the IRF transcription factor family to treat autoimmune and inflammatory diseases.

Materials and Methods

Mice

Irf7^{-/-} transgenic mice were a kind gift from Dr. T. Taniguchi (University of Tokyo, Tokyo Japan) and were backcrossed 10 generations onto C57BL/6 background (14). KRN T cell receptor (TCR) transgenic mice were a gift from Drs. D. Mathis and C. Benoist (Harvard Medical School, Boston, MA) and Institut de Génétique et de Biologie Moléculaire et Cellulaire (Strasbourg, France) (15, 16). C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The mice were bred and maintained under standard conditions in the UCSD Animal Facility accredited by the American Association for Accreditation of Laboratory Animal Care. All animal experiments were carried out according to protocols approved by the Institutional Animal Care and Use Committee.

Passive K/BxN serum transfer arthritis

Arthritic adult K/BxN mice were bled and the sera were pooled. Groups of 8 to 10 recipient mice were injected with 75 μ L pooled sera intraperitoneally (IP) on day 0 and day 2. The standard 150 μ L amount was decreased 75 μ L to allow detection of the clinical effect. Some groups also received 1000 IU of intraperitoneal IFN β (Chemicon, Temecula, CA) daily, 200 μ g of poly (I-C) (Sigma, St. Louis, MO) on day 0 and day 2, or PBS. Clinical arthritis scores were evaluated using a scale of 0–4 for each paw for a total of 16. Ankle thickness was measured with a caliper (Mitutoyo Corporation, Japan) in mm.

Microcomputed tomography (micro-CT) of the calcaneus

Ankle joints of arthritic WT and *Irf7*^{-/-} mice were imaged on day 12 using the Skyscan 1076 microCT-40 system (Kontich, Belgium). Samples were scanned at 9 μ m voxel size, applying an electric potential of 50 kVp and current of 200 μ A, using a 0.5mm aluminum filter. Skyscan software, Dataviewer, CTAn, CT Vol (Kontich, Belgium) was used for orientation and analysis to determine bone histomorphometric parameters. Cortical and trabecular bone analysis was performed on the calcaneus.

Gene Expression

The ankles of arthritic mice were snap frozen and pulverized. FLS and macrophage mRNA from non-arthritic mice was isolated with RNA STAT-60 (Tel Test Inc., Friendswood, TX). Total RNA isolation with RNA STAT and cDNA synthesis by RT-PCR were performed as previously described (17). Forward and reverse primers as well as fluorogenic TaqMan FAM/TAMRA-labeled hybridization probes were used for IFN β , TGF β , IL-1RA, IL-6, RANTES, MIP-1 α , IFN γ -inducible protein 10 (IP-10), matrix metalloproteinase-3 (MMP-3), and vascular endothelial grow factor alpha (VEGF α) (Assay on Demand; Applied Biosystems, Foster City, CA) and normalized to hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT). Data are expressed as the ratio between gene of interest CE and HPRT1 CE, yielding the relative gene expression (RE).

Cytokine Measurement

Serum from whole blood was obtained from wild type (WT) and *Irf7*^{-/-} mice and assayed for IFN β , IL-1RA, and IL-10 using a commercial capture ELISA (R&D Systems, Minneapolis, MN).

Murine synoviocytes and peritoneal macrophages

FLS and peritoneal macrophages were isolated from IRF7 deficient mice. We derived murine *Irf7*^{-/-} FLS from these animals by dissecting synovium, mincing the tissue and enzymatically dispersing the cells with 1 mg/ml collagenase type VIII in serum-free RPMI 1640 for 1 hour at 37 $^{\circ}$ C. FLS were used from passage 3 through 8 during which time they were a homogeneous population of cells (<1% CD11b positive, <1% phagocytic, and <1% FcR II and FcR III receptor positive) (12). In addition, we isolated peritoneal macrophages via intraperitoneal lavage with PBS. FLS and macrophages were stimulated with 10 μ g/mL poly (I-C) (Sigma, St. Louis, MO) for 18h. Cells were harvested with 1mL RNA STAT-60 (Tel Test Inc., Friendswood, Texas, USA) per sample.

Statistics

Data are expressed as a mean \pm SEM. Comparisons of PCR gene expression data and ELISA cytokine measurements were performed by Student's *t* test. Areas under the curve were calculated from delta paw swelling. Two-way ANOVA was used for multiple comparisons. P values of < 0.05 were considered statistically significant.

Results

IRF7 deficiency increases the clinical severity of K/BxN serum transfer arthritis

The type I IFN response plays a critical role in inflammation and is regulated by several transcription factors as part of the IFN enhanceosome (18). To evaluate the *in vivo* contribution of IRF7 to synovial inflammation and arthritis, we used the passive transfer K/BxN arthritis model in *Irf7*^{-/-} mice. Deficiency of IRF7 resulted in increased arthritis in the K/BxN antibody-mediated passive transfer model after day 8 (Figure 1A) shown as change in paw swelling in *Irf7*^{-/-} and WT mice (average change in paw measurement in mm). On days 8 and 12, there was increased arthritis observed in the *Irf7*^{-/-} mice suggesting a counter-regulatory or anti-inflammatory role for IRF7 in this model. Arthritis severity in *Irf7*^{-/-} mice was significantly increased on day 8 (average change in paw measurement 5.5mm WT vs. 9.9mm *Irf7*^{-/-}, $p < 0.02$) and on day 12 (3.5mm WT vs. 8.6mm *Irf7*^{-/-}, $p < 0.01$) compared with WT (Figure 1A, left). Total area under the curve (AUC) was significantly increased in *Irf7*^{-/-} mice compared with WT (Figure 1A, right, $p < 0.05$). No significant difference in synovial histology was detected in the *Irf7*^{-/-} ankle tissue compared with WT (data not shown). The lack of effect on synovial histology despite increased paw volume suggested that IRF7 might also play a role in vascular permeability and subsequent tissue edema contributing to paw measurement differences as noted in other arthritis models (19, 20). In addition, clinical score and paw measurements are more sensitive compared with histological score so it might be difficult to detect a significant difference with this degree of arthritis and joint inflammation.

Augmented systemic and local pro-inflammatory cytokine profiles in arthritic *Irf7*^{-/-} mice

To evaluate potential mechanisms for increased arthritis in the *Irf7*^{-/-} K/BxN passive transfer model, we analyzed synovial tissue extracts from ankle joints of the mice on day 4 of the arthritis model (Figure 1B). Most notably and in agreement with the clinical effect of increased arthritis, IFN β gene expression was significantly less in IRF7 deficient synovium. Gene expression of IFN β , RANTES, MIP1 α , and IL-6 was decreased in K/BxN *Irf7*^{-/-} synovial tissue compared with K/BxN WT synovium (3.9-fold, $p < 0.05$; 2.2-fold, $p < 0.05$; 2.4-fold, $p < 0.04$; 1.7-fold, $p < 0.05$ respectively). Gene expression of MMP3 and IP-10 was not significantly different in ankle *Irf7*^{-/-} synovial tissue compared with WT. Of note, the significant decrease of IFN β mRNA in the ankle tissue of the *Irf7*^{-/-} mice suggested that the exacerbation of arthritis could be due to lower levels of this anti-inflammatory cytokine in mouse arthritis models. Expression of other anti-inflammatory genes, including IL-1RA and TGF β , as well as VEGF α , was not significantly different compared with control arthritic synovial tissue.

To further investigate the possibility that IRF7 deficiency exacerbates K/BxN arthritis by decreasing type I IFN, serum levels of the anti-inflammatory cytokine IFN β were evaluated. In agreement with decreased synovial IFN β gene expression in arthritic *Irf7*^{-/-} K/BxN mice, serum IFN β levels were also significantly decreased in *Irf7*^{-/-} mice (2.8-fold, $p < 0.04$) compared with WT K/BxN (Figure 1C). Therefore, decreased IFN β in serum and synovial tissue might contribute to the increase in K/BxN arthritis in *Irf7*^{-/-} mice. Decreased expression of the anti-inflammatory cytokine IFN β in both synovium and serum is supportive of the overall clinical effect of increased arthritis. Serum levels of IL-10 and IL-1RA were not significantly different in *Irf7*^{-/-} mice compared with WT.

Analysis of bone damage by micro-CT image analysis

Because histologic evaluation the degree of extra-articular swelling in the tissue sections revealed no significant difference between WT and *Irf7*^{-/-}, we performed micro-CT to further evaluate tissue and bone damage in the arthritic animals. We used micro-CT to

determine differences in cortical and trabecular bone damage between WT and *Irf7*^{-/-} arthritic serum transfer mice (Figure 2). Three-dimensional reconstruction of the calcaneus showed no significant difference in cortical bone area/total area (BA/TA) computed as percent object area for WT (79.3 ± 3.0) compared with *Irf7*^{-/-} (79.5 ± 2.1). In addition, no significant difference was detected in trabecular bone volume/tissue volume (BV/TV) computed as percent bone volume in arthritic WT (20.8 ± 3.4) compared with *Irf7*^{-/-} mice (21.6 ± 3.4). This data supported the histological analysis that revealed no difference in bone erosions in WT compared with *Irf7*^{-/-} arthritic mice.

IFN β treatment suppresses *Irf7*^{-/-} K/BxN inflammatory arthritis

To explore the role of IFN β in regulation of *Irf7*^{-/-} K/BxN inflammatory arthritis, mice were treated with replacement recombinant IFN β or PBS (Figure 3A). *Irf7*^{-/-} mice treated with IFN β demonstrated an average relative decrease in paw swelling similar to IFN β treated WT mice, suggesting that the degree of arthritis inhibition from baseline by IFN β treatment was similar and the treatment effectively decreased arthritis. The severity of arthritis by total area under the curve for *Irf7*^{-/-} mice was decreased to the level of untreated WT by replacement IFN β treatment (Figure 3A). The relative mean decrease was 47% and 36% for WT and *Irf7*^{-/-} mice, respectively (p<0.05). Hence, the clinical effect of IFN β treatment did not require IRF7 and was likely due to the direct anti-inflammatory effect of IFN β treatment. Arthritis was significantly increased in *Irf7*^{-/-} mice compared with WT mice treated with IFN β (p<0.05).

IFN β treatment increases serum IL-1RA levels in IKK ϵ mice (10), suggesting that IFN β enhances the anti-inflammatory effect of IKK ϵ deficiency through induction of IL-1RA. These results supported other studies demonstrating that the anti-inflammatory IFN β mechanism of action requires IL-1RA (5, 21). To evaluate this potential mechanism in the *Irf7*^{-/-} mice, IL-1RA ELISA was performed on serum from *Irf7*^{-/-} K/BxN mice compared with control serum (Figure 3B). In contrast to control PBS, IFN β treatment of *Irf7*^{-/-} mice resulted in a significant increase in serum IL-1RA levels. Therefore, IFN β treatment induced serum IL-1RA production in the *Irf7*^{-/-} mice compared with control. This effect, as well as the direct anti-inflammatory effect of IFN β in mouse arthritis models, might contribute to the decrease in arthritis with IFN β treatment.

Poly (I-C) treatment decreases passive K/BxN arthritis in *Irf7*^{-/-} mice

To determine the role of IRF7 in the poly (I-C) mediated inhibitory effect on serum transfer arthritis (9, 10), we performed the passive model in *Irf7*^{-/-} and WT mice treated on days 0 and 2 with poly (I-C). Arthritis severity was significantly decreased in poly (I-C) treated *Irf7*^{-/-} mice relative to PBS treated mice (Figure 4A). Because treatment with poly (I-C) decreased paw swelling in *Irf7*^{-/-} mice compared with PBS control, other IRF or transcription factors likely play a partial role in poly (I-C) mediated inhibition of arthritis. Despite the poly (I-C) treatment, overall arthritis remained increased in the *Irf7*^{-/-} mice compared with WT.

Next, we evaluated changes in synovial tissue gene expression with poly (I-C) treatment. Figure 4B shows gene expression in ankle synovial tissue from poly (I-C) treated *Irf7*^{-/-} K/BxN compared to poly (I-C) treated WT mice. Comparison of the standard serum transfer model synovial PCR (Figure 1B) with the *in vivo* poly (I-C) treated arthritis model PCR (Figure 4B) we noted a significant change in IFN β gene expression. *In vivo* poly (I-C) treatment of *Irf7*^{-/-} mice restored IFN β gene expression (decreased WT IFN β at baseline shown in Figure 1B), in synovial tissue to WT levels (Figure 4B), suggesting that the effect of poly (I-C) might occur via induction of IFN β by other IRF family members. Poly (I-C) treatment did not induce a significant difference in gene expression of the anti-inflammatory

cytokines IL-1RA and TGF β in K/BxN *Irf7*^{-/-} synovium. Induction of IL-6 gene expression was significantly less in poly (I-C) treated *Irf7*^{-/-} synovial tissue compared with poly (I-C) treated WT; however, IL-6 is not an absolute requirement for arthritis in this serum transfer model (22). Induction of RANTES was increased in the *Irf7*^{-/-} synovial tissue after *in vivo* poly (I-C) treatment, opposite of the clinical effect. Figure 4C summarizes and compares the different effects of poly (I-C) treatment on IFN β synovial gene expression in *Irf7*^{-/-} mice compared with WT. Poly (I-C) treatment of *Irf7*^{-/-} mice resulted in 3.6-fold induction of IFN β gene expression compared with control PBS ($p < 0.001$), but had no significant effect on WT IFN β gene expression in the synovium. Of note, the baseline gene expression of IFN β is decreased in the *Irf7*^{-/-} mice and poly (I-C) treatment restores mRNA to WT levels. IFN β protein expression was also increased in the serum of *Irf7*^{-/-} K/BxN mice treated with *in vivo* poly (I-C) compared with PBS control (Figure 4D). Serum IFN β was significantly increased by poly (I-C) in *Irf7*^{-/-} mice suggesting that serum IFN β levels correlate with decreased arthritis in this model. Baseline serum IFN β protein concentration was decreased in *Irf7*^{-/-} mice compared with WT.

Increased induction of poly (I-C) stimulated pro-inflammatory gene expression in *Irf7*^{-/-} FLS

To dissect cell specific roles of IRF7 in arthritis, we performed *in vitro* poly (I-C) stimulation of murine FLS lines and peritoneal macrophages derived from non-arthritic *Irf7*^{-/-} mice. No significant difference in IFN β gene induction was detected in *Irf7*^{-/-} FLS. Poly (I-C) stimulation of *Irf7*^{-/-} FLS resulted in increased induction of gene expression of RANTES (5.2-fold, $p < 0.01$), MIP1 α (1.8-fold, $p < 0.05$), and MMP-3 (2.7-fold, $p < 0.05$) compared with WT FLS (Figure 5A). Induction of IP-10 gene expression was decreased in the *Irf7*^{-/-} FLS in response to poly (I-C) stimulation. Taken together, these results suggest that IRF7 might play a counter regulatory or anti-inflammatory role in poly (I-C) induced responses in murine FLS independent of IFN β .

Decreased induction of IFN β gene expression in *Irf7*^{-/-} macrophages

Because IRF7 did not contribute to IFN β production in FLS, we evaluated the role of IRF7 in poly (I-C) induced IFN β production by macrophages. To evaluate the effect of genetic deficiency of IRF7 in macrophages, we isolated cells from the peritoneal cavity and stimulated *in vitro* with poly (I-C) (Figure 5B). In contrast to FLS, *Irf7*^{-/-} macrophages stimulated with poly (I-C) showed significantly less induction of cytokine gene expression of IFN β compared with WT macrophages (8.8-fold decrease, $p < 0.01$). These results are different from *Irf7*^{-/-} FLS indicating a cell specific regulatory role and suggesting that IRF7 activates poly (I-C)-induced IFN β gene expression in the hematopoietic macrophage subset. IRF7 regulates IFN β gene expression in murine macrophages, but not in stromal FLS.

Discussion

RA is a destructive, inflammatory arthritis that involves activation of innate immunity. Although rheumatoid arthritis cannot be directly studied in mice, the passive serum transfer model is useful because of its simplicity and primary dependence on activation of innate immunity (23). This passive model of arthritis can be studied in genetically deficient mice to assess pathogenic mechanisms of innate immunity in inflammatory arthritis (24). In addition, TLR signaling through MyD88 has been implicated in the pathogenesis (25). The model requires IL-1, has modest dependence on TNF α , and pertinent to the IRF7 arthritis to be discussed, does not require IL-6 (22, 26). This model provides insights that are useful to understanding innate immune activation and effector mechanisms of IRF7 and type I IFN regulation in inflammatory arthritis.

We hypothesized that IRF7 differentially regulates synovial inflammation in a cell specific manner that can alter innate immunity and the clinical course of arthritis *in vivo*. To screen for a functional effect of IRF7 deficiency in arthritis, we evaluated the serum transfer model of antibody-mediated arthritis in mice. Genetic IRF7 deficiency exacerbated arthritis suggesting that IRF7 plays an overall counter-regulatory role in the later stages of serum transfer K/BxN arthritis. However, in light of the lack of a significant difference in histologic score evaluating bone erosion, cartilage erosion, and synovial inflammation, the change in paw volume might be related to vascular permeability and a difference in extra-articular swelling. The lack of effect on synovial histology despite increased paw volume suggested that IRF7 might also play a role in vascular permeability and subsequent tissue edema contributing to paw measurement differences as noted in other arthritis models (19, 20). Of note, several viruses alter vascular permeability in endothelial cells through cytokine expression as a mechanism of infection during human disease. IRF7 could contribute to regulation of cytokine expression and increased vascular permeability in this arthritis model.

Because IRF7 regulates type I IFN responses and IFN β expression was decreased in the serum and synovium of *Irf7*^{-/-} mice, we also hypothesized that the increase in arthritis in the *Irf7*^{-/-} mice could be partially due to an insufficiency of IFN β . To explore the role of IRF7 in the response to IFN β , *Irf7*^{-/-} mice were injected with replacement IFN β concurrently with serum transfer. IFN β treatment of *Irf7*^{-/-} mice resulted in a decrease in arthritis to similar to WT PBS treated mice. Although the *Irf7*^{-/-} mice were not able to mount an endogenous IFN β response during the course of arthritis, they were able to respond adequately to the exogenous IFN β treatment. Exogenous IFN β treatment also enhanced serum IL-1RA production that could have also contributed to the beneficial effects of this treatment in the *Irf7*^{-/-} mice, which is consistent with other reports (5, 21). More specifically, the mechanism of the beneficial effects of IFN β in passive K/BxN arthritis are dependent on IL-1RA in this mouse model of arthritis (27).

Although IRF7 decreases serum transfer arthritis, the upstream kinase IKK ϵ that activates IRF7 actually increases arthritis in this model. We proposed that IRF7 decreases arthritis through production of IFN β , an anti-inflammatory cytokine. The initial type I IFN response to innate receptor activation results in IKK ϵ /TBK1 phosphorylation of IRF3 and IRF7. This signaling induces type I IFN that bind the IFN α/β receptor and further induce IRF7 transcription and also secondary activation of IKK ϵ (28). Therefore, an amplification loop can proceed through the IKK-related kinases, especially IKK ϵ (29). Certain type I IFN-stimulated genes are not activated in the absence of IKK ϵ because the IFN-stimulated gene factor 3 (ISGF3) complex does not bind to promoter of those particular genes. This pathway involves IFN β mediated activation of IKK ϵ , followed by IKK ϵ phosphorylation of signal transducer and activator of transcription 1, a component of ISGF3. This might contribute to the opposite clinical effect of IRF7 and IKK ϵ in the serum transfer arthritic mice.

TLR activation has been shown to contribute to inflammation in autoimmune diseases; however, repeated exposure to a TLR agonist can induce a hypo-responsiveness or desensitization to further TLR stimulation (30). The TLR3 agonist poly (I-C) has been previously studied in the K/BxN passive antibody-mediated arthritis model in WT and in IKK ϵ null mice and resulted in decreased arthritis if co-administered at the onset of disease with serum (10). Serum levels of IL-1RA were increased in IKK ϵ null IFN β treated mice, which was proposed to contribute to the clinical decrease in arthritis. IL-1RA mediates the beneficial effect of systemic IFN β therapy in the serum transfer model (27). Because poly (I-C) induces IRF7 *in vitro*, we used *Irf7*^{-/-} mice to determine if the poly (I-C)-mediated decrease in serum transfer arthritis is dependent on IRF7 mediated induction of an anti-inflammatory type I IFN response. Surprisingly, poly (I-C) treatment also decreased arthritis in *Irf7*^{-/-} arthritic mice to the same degree as the WT mice. The partial effect might have

occurred through inducing and restoring synovial IFN β gene expression to WT levels. However, the IFN effect might be correlative rather than causal to changes in arthritis. The functional role of synovial IFN β is not definitive because the WT mice treated with poly (I-C) have reduced arthritis without an increase in synovial IFN β . More importantly and consistent with the clinical response, serum IFN β protein levels in *Irf7*^{-/-} mice were also increased by poly (I-C) treatment. This indicated that other IRF or transcription factors might have contributed to poly (I-C)-mediated inhibition of arthritis in the *Irf7*^{-/-} mice and that circulating IFN β likely plays a more important role compared with synovial gene expression.

The *Irf7*^{-/-} mice exhibited an accentuated clinical course in the passive serum transfer model, yet were able to respond to both the poly (I-C) and IFN β treatments *in vivo*. Whole joint preparations demonstrated a reduction in the overall level of IFN β mRNA transcription, which might reflect the cellular composition of FLS and macrophage-like synoviocytes in the swollen joints. We have studied FLS in RA and mouse models of arthritis and propose that they are important players in inflammatory arthritis. In RA, FLS display a transformed phenotype and also express cadherin-11, an adhesion molecule essential for the development of the synovium and thickening of the lining (31). Although many cell types can contribute to the vicious cycle that initiates and perpetuates inflammatory arthritis, we included FLS and macrophage data to accompany the *in vivo* studies. Similar to human FLS knockdown studies indicating a minimal role for IRF7 in FLS IFN production (13), no significant difference in IFN β gene expression was detected in poly (I-C) stimulated *Irf7*^{-/-} FLS. In contrast, IRF7 regulated the induction of several IFN-stimulated genes including RANTES, IP-10, MIP1 α , as well as MMP3 in mouse FLS in response to poly (I-C). These results suggest that IRF7 might play a counter regulatory or anti-inflammatory role in poly (I-C)-induced responses in murine FLS independent of IFN β .

Because the *Irf7*^{-/-} FLS were able to generate IFN β transcripts in response to *in vitro* poly (I-C) stimulation (Figure 5A), the lack of IFN β expression could not be attributed to an inherent deficit produced by FLS. Hence, to further examine the reduction in IFN β seen in the arthritic *Irf7*^{-/-} synovium, we evaluated gene expression in macrophages stimulated with poly (I-C). In contrast to FLS, IRF7 deficient macrophages showed significantly less induction of IFN β gene expression in response to poly (I-C). These results suggested a cell specific, key regulatory role for IRF7 in IFN β production by the macrophages, but not in stromal FLS. It is uncertain whether the *in vitro* profile of *Irf7*^{-/-} peritoneal macrophages stimulated with poly (I-C) plays any role in synovial IFN β gene expression in the serum transfer model. The overall pro-inflammatory profile of FLS combined with the loss of macrophage anti-inflammatory IFN β might act together to increase arthritis in the *Irf7*^{-/-} K/B \times N mouse model. *In vitro* studies in human monocytic cell lines confirm these cell specific roles for IRF in IFN-regulated gene expression (in press).

IFN β plays a complex role and suppresses inflammation, especially in mouse models of arthritis. Mice with collagen-induced arthritis injected with fibroblasts expressing IFN β have decreased arthritis severity and bone and cartilage destruction (5). An alternative approach in the passive serum transfer model combined IKK ϵ deficiency with subtherapeutic IFN β , which amplified the anti-inflammatory effects of this pathway (10). IRF7 plays a counter regulatory role in the K/B \times N serum transfer model of arthritis likely through cell specific effects on IFN production and cytokine gene expression. Thus, careful dissection of the signaling pathways that regulate the type I IFN response and identification of the key regulatory transcription factors, kinases, and IFN-response genes could shed light on new ways to modulate anti-inflammatory effects of this pathway without markedly suppressing host defense in RA patients.

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Abbreviations

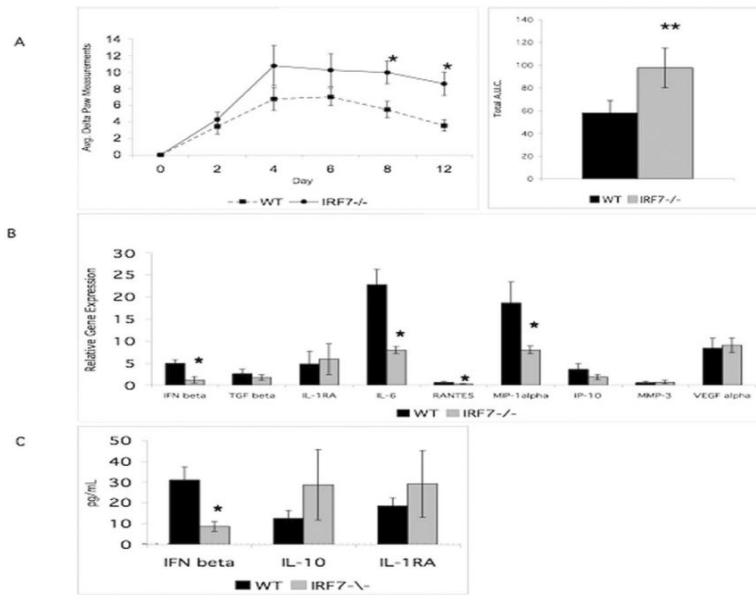
FLS	fibroblast-like synoviocyte
HPRT	hypoxanthine-guanine phosphoribosyltransferase
IKK	I κ B kinase
IL-1RA	IL-1 receptor antagonist
IP-10	IFN inducible protein 10
IP	intraperitoneal
IRF	IFN regulatory factor
MMP	matrix metalloproteinase
poly I-C	polyinosinic polycytidylic acid
RA	rheumatoid arthritis
RE	relative expression
WT	wild type

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**Figure 1.**

Irf7^{-/-} mice have increased arthritis in the passive K/BxN serum transfer model. A. *Irf7*^{-/-} mice developed significantly more paw swelling than WT controls day 8 and day 12 (left panel, *p<0.05). Total area under the curve (AUC) was significantly increased in *Irf7*^{-/-} mice compared with WT (right panel, **p<0.05). Data are representative of 2 independent experiments. B. IFN β was decreased in *Irf7*^{-/-} synovial tissue. Relative expression (RE) of IFN β , IL-6, RANTES, and MIP-1 α was decreased (*p<0.05) compared with control. RE of TGF β , IL-1RA, IP-10, MMP-3, and VEGF α was not significantly different. C. Serum IFN β protein measured by ELISA was decreased in the *Irf7*^{-/-} arthritic mice (*p<0.009, n=8 mice per group). Levels of IL-10 and IL-1RA were not different in sera of WT and *Irf7*^{-/-} K/BxN mice.

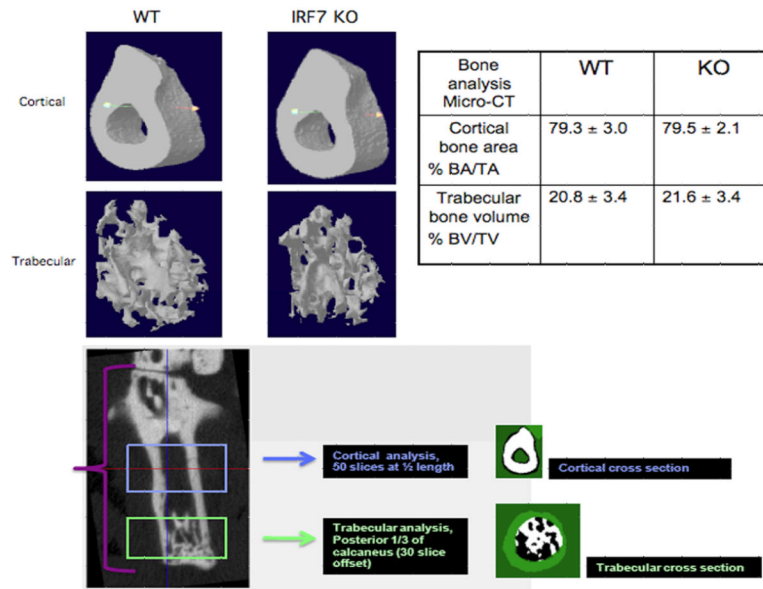


Figure 2. Visualization, 3D reconstruction, and quantification of serum transfer induced joint damage by microcomputed tomography. Representative images of arthritic WT and *Irf7*^{-/-} mice (n=5 each) cortical and trabecular bone are shown in upper left images. Percent cortical bone area and trabecular bone volume calculations are shown in the table on the upper right. No significant difference between WT and IRF7 KO cortical bone area or trabecular bone volume was detected. The lower image demonstrates the regions of micro-CT used for cortical and trabecular bone analysis.

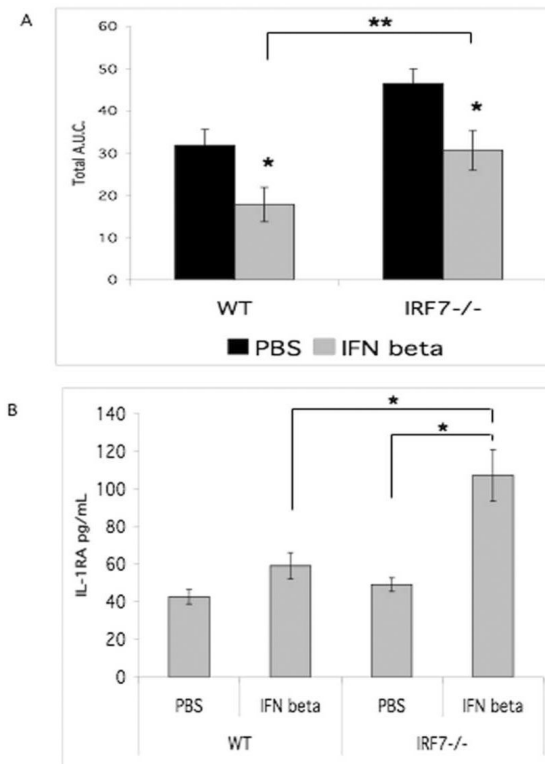
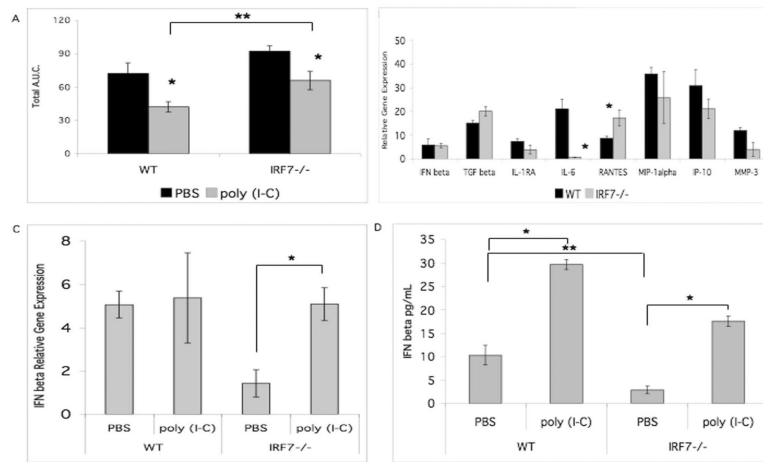


Figure 3.

IFN β treatment in *Irf7*^{-/-} mice effectively decreased arthritis. A. *Irf7*^{-/-} and WT mice were injected with serum on day 0 and 2. Mice were injected IP daily with IFN β 1000 IU or PBS. Treatment of the *Irf7*^{-/-} mice with replacement IFN β decreased the amount of arthritis to WT levels. The inhibition of arthritis by IFN β treatment in *Irf7*^{-/-} mice was significant and IFN β treatment also decreased arthritis in WT mice (* p <0.05). Arthritis was significantly increased in *Irf7*^{-/-} mice despite IFN β treatment (** p <0.05). B. Serum IL-1RA was increased in *Irf7*^{-/-} mice treated with IFN β compared with control treatment (* p <0.03). IL-1RA was significantly higher in *Irf7*^{-/-} serum compared with WT after treatment with IFN β (* p <0.03).

**Figure 4.**

Treatment with poly (I-C) decreased arthritis in the passive serum transfer model. A. *Irf7*^{-/-} and WT mice were injected with serum on day 0 and 2. Treatment was performed with IP injection of poly (I-C) or PBS on day 0 and 2. Both WT and *Irf7*^{-/-} poly (I-C) treatment groups had less paw swelling (*p<0.05). Treatment with poly (I-C) decreased the amount of arthritis in *Irf7*^{-/-} to WT levels. *Irf7*^{-/-} mice had increased arthritis compared with WT despite treatment with poly (I-C) (**p<0.05). B. Poly (I-C) mediated induction of gene expression in the synovial tissue of *Irf7*^{-/-} mice demonstrated that IFNβ gene expression was restored to WT levels. C. Poly (I-C) treatment in *Irf7*^{-/-} mice increases IFNβ gene expression to WT levels (*p<0.001). D. IFNβ protein by ELISA was increased in the sera of arthritic WT and *Irf7*^{-/-} mice treated with poly (I-C) (*p<0.05). Baseline IFNβ in serum is decreased in *Irf7*^{-/-} mice (**p<0.05).

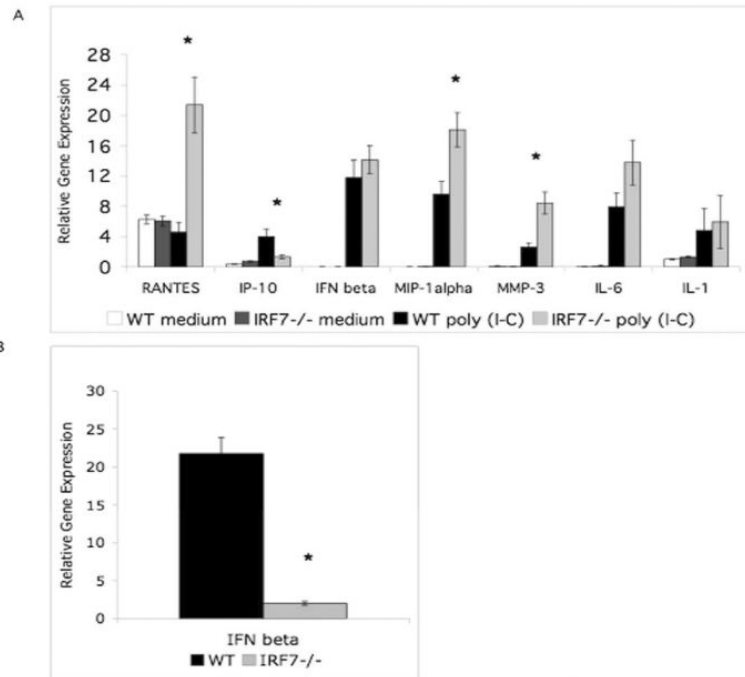


Figure 5.

Poly (I-C) induced gene expression of murine *Irf7*^{-/-} FLS and peritoneal macrophages. Mouse *Irf7*^{-/-} FLS and peritoneal macrophages were stimulated with poly (I-C). QPCR analysis of cytokine gene expression was determined for RANTES, IP-10, IFN β , MIP-1 α , MMP-3, and IL-6 normalized to HPRT1. A. Poly (I-C) stimulation of *Irf7*^{-/-} FLS resulted in increased induction of several pro-inflammatory cytokines compared with control (*p<0.05). B. Poly (I-C) stimulation of *Irf7*^{-/-} peritoneal macrophages resulted in significantly less induction of IFN β gene expression compared with control (*p<0.05).