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Suppression of the Effector Phase of Inflammatory Arthritis by Double-Stranded RNA Is Mediated by Type I IFNs

Anna Yarilina,* Edward DiCarlo,† and Lionel B. Ivashkiv2*‡

Innate immune receptors that recognize nucleic acids, such as TLRs and RNA helicases, are potent activators of innate immunity that have been implicated in the induction and exacerbation of autoimmunity and inflammatory arthritis. Polyriboinosine-polyribocytidylic acid sodium salt (poly(IC)) is a mimic of dsRNA and viral infection that activates TLR3 and the RNA helicases retinoic acid-induced gene-1 and melanoma differentiation-associated gene-5, and strongly induces type I IFN production. We analyzed the effects of systemic delivery of poly(IC) on the inflammatory effector phase of arthritis using the collagen Ab-induced and KRN TCR-transgenic mouse serum-induced models of immune complex-mediated experimental arthritis. Surprisingly, poly(IC) suppressed arthritis, and suppression was dependent on type I IFNs that inhibited synovial cell proliferation and inflammatory cytokine production. Administration of exogenous type I IFNs was sufficient to suppress arthritis. These results suggest a regulatory role for innate immune receptors for dsRNA in modulating inflammatory arthritis and provide additional support for an anti-inflammatory function of type I IFNs in arthritis that directly contrasts with a pathogenic role in promoting autoimmunity in systemic lupus. The Journal of Immunology, 2007, 178: 2204–2211.

However, TLRs also engage potent feedback inhibitory and homeostatic mechanisms. Most TLRs induce production of IL-10 that limits the extent of inflammation and associated tissue damage (10) and can modulate immune responses against pathogens. For example, TLR2 induces high levels of IL-10 production that suppress immune responses against bacterial infections (11, 12). TLRs that recognize LPS (TLR4) or nucleic acids (TLR3, TLR7, TLR8, and TLR9) are strong inducers of the pleiotropic type I IFNs. In contrast to other TLRs, TLR3, the receptor for dsRNA, does not engage the MyD88-dependent signaling pathway and is a relatively weak inducer of inflammatory cytokines such as TNF and IL-1. Instead, TLR3 mediates innate responses against dsRNA viruses by inducing production of large amounts of type I IFNs. Type I IFNs are also strongly induced when retinoic acid-induced gene-1 (RIG-1) and melanoma differentiation-associated gene-5 (Mda5)-signaling pathways are triggered by dsRNA (3).

Type I IFNs (IFN-α and -β) are pleiotropic cytokines that can be produced by many cell types in response to viral, bacterial, and inflammatory stimuli and IFN itself (13). Type I IFNs have multiple, sometimes opposite, effects depending on their dose, local or systemic administration, cell type, and state of differentiation. Besides their originally described potent antiviral effects, type I IFNs regulate cell growth and differentiation and modulate immune and inflammatory responses (14, 15). Type I IFNs promote immunity via stimulation of chemokine and cytokine production, enhancement of dendritic cell maturation and function, up-regulation of MHC class I and II expression, induction of NK and T cell cytotoxicity, and promotion of Th1 responses, B cell class switching, and Ab production (15). Alternatively, type I IFNs also can suppress immunity and inflammation. For example, type I IFNs can decrease delayed type hypersensitivity, suppress proliferative responses of T cells to mitogens, and suppress endotoxin-induced mortality under certain conditions (16). Human diseases that have been reported to benefit from type I IFN treatment include multiple sclerosis, Sjögren’s syndrome, idiopathic thrombocytopenic purpura, inflammatory bowel disease, Behçet’s disease, chronic recurrent multifocal osteomyelitis, and some tumors (15, 17–21). The mechanisms underlying the beneficial effects of type I IFNs are not well understood and include suppression of cell proliferation and...
growth arrest; inhibition of IL-12, IL-1, and TNF-α production and activity; and, under specific conditions, suppression of IFN-γ production (15, 16, 22).

TLRs, including TLR2, TLR3, TLR4, are expressed in joint synovium, and there is an emerging interest in understanding the role of TLRs in the pathogenesis of inflammatory arthritis. Local synovial injection of TLR2, TLR3, TLR4, and TLR9 ligands has been clearly shown to induce or exacerbate arthritis (5, 8, 23, 24). In our study, we investigated the effects of systemic administration of dsRNA on experimental arthritis. We took advantage of two models of experimental arthritis that bypass the need to induce an autoimmune response by administering autoantibodies that directly induce immune complexes in joints and thereby induce synovial inflammation. This allowed us to test the effects of dsRNA solely on the inflammatory effector phase of arthritis. Surprisingly, dsRNA suppressed arthritis in a manner that was dependent on type 1 IFNs and suppression of synovioctye proliferation and of inflammatory cytokine production. These results suggest that activation of TLRs or related signaling pathways downstream of RIG-I-LDmd5 is not always detrimental for inflammatory disease pathogenesis, and they provide additional evidence supporting an anti-inflammatory role for type 1 IFNs in arthritis.

Materials and Methods

Reagents
Polyribinosine-polyribocytidylic acid sodium salt (poly(IC)) was purchased from Sigma-Aldrich, Arthrogen-CIA arthritis-inducing mAb mixture and Escherichia coli LPS was from Chemicon International (Temecula, CA), and IFN-α/β, a universal mammalian type 1 IFN, was from R&D Systems, rabbit monoclonal anti-Ki67 Ab was from NeoMarkers (Lab Vision), Vectastain ABC kit, Vector NovaRED substrate, and Ag Unmasking Solution were from Vector Laboratories, control rabbit IgG was from Santa Cruz Biotechnology, and Contrast GREEN Solution was from Kirkegaard & Perry Laboratories.

Mouse and induction of experimental arthritis

Wild-type C57BL/6 and NOD/Lt mice were purchased from The Jackson Laboratory, IFNAR1-deficient mice (a gift from Dr. D. Levy, NYU School of Medicine, New York, NY) and genetically matched control mice were maintained on a C57BL/6J × 129/Sv genetic background, KRN TCR-transgenic mice (a gift from Drs. D. Mathis and C. Benoist, Harvard Medical School, Boston, MA) were bred to NOD/Lt mice to generate K/BxN mice. All animals were bred and maintained under specific pathogen-free conditions in the Animal Facility of the Hospital for Special Surgery (New York, NY), and all protocols were approved by the Institutional Animal Care and Use Committee. Collagen Ab-induced arthritis (CAIA) was induced in 6- to 8-wk-old male mice by i.p. administration of 4 mg of Arthrogen-CIA arthritis-inducing mAb mixture at day 0 followed by injection of 50 μg of LPS on day 2 (25). Control animals were injected with saline at day 0 and 50 μg of LPS on day 2. K/BxN serum pools were prepared from adult arthritic mice as previously described (26). Arthritis was induced by i.p. injection of 150 μl of serum at days −2 and 0. Control animals were injected with saline only. The severity of arthritis was monitored by measuring the thickness of both wrist and ankle joints using a type dial caliper (Bel-Art Products). For each animal, the joint thickness was calculated as a sum of measurements of two wrists and two ankles. The joint thickness was represented as an average for every group of treatment.

Cell culture

Murine bone marrow-derived macrophages from C57BL/6 mice were obtained by culturing bone marrow in DMEM supplemented with 20% FBS (HyClone) containing 10 ng/ml murine M-CSF (Peprotech) as described (27). Macrophages at 2 × 10^4 cells/well (six-well plate) were stimulated with 25 μg/ml poly(IC) for 24 h and then with 10 ng/ml LPS for another 24 h.

Histopathology

For histopathology, knee joints were fixed in 10% neutral buffered formalin for 24 h, decalcified with 10% neutral buffered EDTA for 6 days, and embedded in paraffin. Sections 5 μm thick were stained with either H&E or safranin O using standard techniques. Synovial membranes, bones, and cartilage tissues of knee joints were evaluated blindly using a previously validated scoring system (28) by a certified pathologist who specializes in musculoskeletal diseases and independently by a second blinded investi-

The Journal of Immunology

oratory.

Statistical analysis

Statistical analysis was done using SPSS Analytical Software version 8.0.2 for Windows (SPSS). The Mann-Whitney U test was performed to determine whether statistically significant differences existed among the groups.

Immunohistochemistry

Sections were prepared as described for histopathology. Before incubation with Abs samples were microwaved for 5 min in Ag Unmasking Solution and then left to cool to room temperature; endogenous peroxidase was blocked by the glucose oxidase-glucose-sodium azide method (30). To detect cell proliferation, sections were stained with rabbit monoclonal anti-Ki67, followed by incubation with biotinylated secondary Abs, and then visualized using the Vectastain ABC kit and Vector NovaRED substrate (stains nuclei red) according to the manufacturer’s protocol. All sections were counterstained with Contrast GREEN Solution which stains nuclei bluish green. Nonproliferating cells stained light bluish green (Contrast GREEN positive only), whereas proliferating cells stained dark blue/purple (Contrast GREEN and NovaRED positive). For each section, the total number of cells and the number of proliferating cells in three different matched regions of the joint were quantitated using digital image analysis with Bioquant Nova version 5.50.8 software.

Analysis of mRNA levels

RNA was isolated from one knee joint, wrists, ankles, and small paw joints of each animal separately. Briefly, legs were cut close to the joints and cleaned from the skin and muscles, bone marrow was washed out, and joints were opened into guanidine isothiocyanate-containing buffer, vortexed for 1 min, and then frozen at −20°C until used. For quantitative real-time PCR, DNA-free RNA was extracted using a RNeasy Mini Kit (Qia-gen) with DNase treatment, and 0.5 μg of total RNA was reverse transcribed using a First Strand CDNA Synthesis kit (Fermentas). Real time PCR was performed in triplicate using the iCycler iQ thermal cycler and detection system and an iQ SYBR Green Supermix (Bio-Rad). Relative expression was normalized for levels of GAPDH. The primer sequences used were: GAPDH, forward primer (5′-ATCAAGAAGGTTGGAAGCA-3′) and reverse primer (5′-AGCAACCTGTCTCCAGTGT-3′); RANTES, forward primer (5′-TTTGAACACAGGCTTGGTT-3′) and reverse primer (5′-GCATTACGTCCATCGGTCACT-3′); vascular endothelial growth factor (VEGFB), forward primer (5′-TGGATGCTTACCAGGGA-3′) and reverse primer (5′-GTCCACGAGGTTCTCAACT-3′); COX-2, forward primer (5′-ATACGACGACCTCCATCTAC-3′) and reverse primer (5′-CAACGAGTAGCTTGATT-3′); IL-1β, forward primer (5′-AGCAAAAAGCAGCGACTCAT-3′) and reverse primer (5′-TATGCCATGTCGTTCAT-3′); IL-6, forward primer (5′-GGCTTGGTCTGCACTGTC-3′) and reverse primer (5′-AGGTGGGATTCTCA-3′) and reverse primer (5′-AGGCAAGGAGGAGG-3′) and reverse primer (5′-AGGCAAGGAGGAGG-3′).
Results

**Poly(IC) suppresses the effector phase of inflammatory arthritis**

We tested whether poly(IC), a mimic of dsRNA and an activating ligand for TLR3, RIG-I, and Mda5, could suppress the inflammatory effector phase of arthritis using the CAIA model. In this well-established model (9, 25), mice are injected with a single dose of a mAb mixture directed against collagen type II, followed by an LPS injection 2 days later. Arthritis develops rapidly (3–4 days after mAb injection) and reaches a maximum at days 6–7 (25). In our study, we sacrificed mice near the peak of arthritis (day 6) to obtain joints for analysis of histology and gene expression. Control mice that were injected with LPS alone did not develop arthritis, as assessed by measuring joint thickness (Fig. 1A) and histological analysis (Fig. 1B). Arthritis develops rapidly (3–4 days after mAb injection) and reaches a maximum at days 6–7 (25). In our study, we sacrificed mice near the peak of arthritis (day 6) to obtain joints for analysis of histology and gene expression. Control mice that were injected with LPS alone did not develop arthritis, as assessed by measuring joint thickness (Fig. 1A) and histological analysis (Fig. 1B). As expected, arthritis developed rapidly in mice injected with anti-collagen Abs and LPS (Fig. 1A). Systemic administration of poly(IC), given i.p., blocked development of arthritis (Fig. 1A). Histological analysis revealed that poly(IC) suppressed synovial hyperplasia, with decreased numbers of synovial lining cell layers and decreased synovial thickness. Representative knee joint sections are shown in Fig. 1B, and a more thorough and quantitative analysis of the histology is presented below. These results suggested that poly(IC) suppresses the inflammatory effector phase of arthritis in the CAIA model.

In the CAIA model, poly(IC) could potentially suppress either inflammation elicited by synovial anti-collagen Abs, or the effects of LPS (which works in this model by transiently inducing elevated levels of IL-1 and TNF-α (31)). To address whether poly(IC)
suppressed inflammation induced by synovial autoantibodies/immune complexes, we used the K/BxN serum transfer model that does not require injection of LPS or any other exogenous adjuvants (32, 33). In this model, arthritis is induced by transfer of arthritogenic serum derived from arthritic K/BxN mice that express high titers of Abs directed against GPI that is expressed in cartilage and synovium. Treatment with poly(IC) substantially and significantly inhibited arthritis in the K/BxN serum transfer model (Fig. 1C).

These results indicate that poly(IC) can suppress the autoantibody/immune complex-dependent component of inflammatory arthritis. Suppression of arthritis by poly(IC) is mediated by type I IFNs Poly(IC) could suppress arthritis indirectly secondary to induction of type I IFN production. Alternatively, direct ligation of synovial TLR3 and/or RIG-I/Mda5 and activation of downstream pathways and genes other than type I IFNs could contribute to suppression. To differentiate between these two possibilities, we used mice deficient in the IFNAR1 subunit of the type I IFN receptor which were on a mixed 129/Sv × C57BL/6 genetic background (34). Poly(IC) suppressed CAIA in control genetically matched mice on the 129 × C57BL/6 background (Fig. 2, top), albeit to a lesser extent than in C57BL/6 mice (Fig. 1A). In contrast to control mice, poly(IC) did not suppress arthritis in the absence of IFNAR1 and type I IFN signaling (Fig. 2, bottom panel). Instead, arthritis was more severe in IFNAR1 knockout animals (Fig. 2, bottom). This result indicates that suppression of arthritis by poly(IC) was dependent on the induction of endogenous type I IFNs and further supports a role for type I IFNs in the suppression of arthritis. One possible explanation for induction of more severe arthritis by poly(IC) in IFNAR1-deficient mice is that type I IFNs function in a feedback inhibition loop to limit poly(IC)-induced inflammatory cytokine production.

To further confirm the inhibitory role of type I IFN in arthritis, we injected mice with recombinant IFN-α A/D, following the

Table 1. Histopathological analysis of knee joints

<table>
<thead>
<tr>
<th>Histopathological Score</th>
<th>Arthritis</th>
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<tr>
<td></td>
<td>No arthritis</td>
</tr>
<tr>
<td>Synovial tissue</td>
<td></td>
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<tr>
<td>Proliferation of synovial cells</td>
<td></td>
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<tr>
<td>Pannus formation</td>
<td></td>
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<tr>
<td>Inflammatory cell infiltration</td>
<td></td>
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<tr>
<td>Presence of debris in the cavity</td>
<td></td>
</tr>
<tr>
<td>Bone and cartilage tissue</td>
<td></td>
</tr>
<tr>
<td>Destruction of bone tissues</td>
<td></td>
</tr>
<tr>
<td>Increase in osteoclasts</td>
<td></td>
</tr>
<tr>
<td>Ostitis/periostitis</td>
<td></td>
</tr>
<tr>
<td>Degeneration and necrosis of chondrocytes</td>
<td></td>
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<tr>
<td>Proteoglycan depletion</td>
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*Joint histopathology analysis was conducted on synovial, bone, and cartilage tissues of knee joints. Scoring was performed in a blinded manner by two observers.*
same regimen as with poly(IC). Similar to poly(IC), type I IFN reduced joint swelling by suppressing hyperplasia of the synovial lining layer and pannus formation (Fig. 3A and B (inset) and Table I). These results indicate that type I IFNs are sufficient to suppress inflammatory arthritis.

It was recently reported that uric acid administered i.p. suppressed arthritis by skewing inflammation from the joint cavity to the site of injection (35). We investigated the possibility that i.p. administration of poly(IC) or IFN-α/H9251 could attenuate arthritis by inducing inflammatory exudates in the peritoneum, thereby diverting inflammation from the joint to the peritoneum. Mice were injected with poly(IC) or with same volume of PBS i.p. every other day, following the same protocol that was used in the arthritis induction experiments. Peritoneal cells were harvested 2 and 4 days after initiation of injections, time points that correspond to the time of initiation of arthritis and the early stage of arthritis where poly(IC) was suppressive (Fig. 1). Poly(IC) did not increase peritoneal cell numbers relative to PBS-treated controls (Fig. 4). We have previously reported that i.p. administration of IFN-α did not induce inflammatory exudates in C57BL/6 mice (36). These results suggested that factors other than skewing of inflammation to another compartment contributed to the suppressive effects of poly(IC) and IFN-α on arthritis.

Next, we examined the effect of poly(IC) on synovial histology. Slides were reviewed blindly by a pathologist who specializes in the musculoskeletal system and arthritis, and a semiquantitative evaluation of histopathology was performed by two independent blinded observers (Table I). Poly(IC) suppressed synovial hyperplasia and pannus formation and also attenuated the extent of tissue damage (Table I). Poly(IC)- and IFN-α-mediated suppression of synovial hyperplasia was more precisely quantitated, and the difference relative to untreated arthritic joints was statistically significant (p < 0.01) for poly(IC) treatment (Fig. 5A). However, although the extent of synovial lining hyperplasia and the size of the inflammatory lesion (pannus) were smaller in poly(IC)- and IFN-α-treated mice, the intensity of inflammatory cell infiltrates was minimally affected by IFN-α and poly(IC) (Figs. 1B and 3B and Table I). Thus, IFN-α and poly(IC) attenuated arthritis by diminishing the amount of inflammatory tissue, but not changing the intensity of inflammatory infiltrates.

The combination of comparable intensity of inflammatory infiltrates with less synovial hyperplasia and pannus formation suggested that IFN-α and poly(IC) may suppress arthritis, at least in

**FIGURE 4.** Local effects of poly(IC) at the site of injection. Poly(IC) does not induce peritoneal inflammation. C57BL/6 mice were injected with 150 μg of poly(IC) in 500 μl of PBS or with PBS alone. Mice were sacrificed on days 2 and 4 after the first injection. Peritoneal cells were washed out with saline and counted. Data are the results of two independent experiments (total of eight animals per group of treatment for each day).

**FIGURE 5.** Poly(IC) and IFN-α suppress synovial proliferation and pannus formation. A, Synovial lining thickness. Knee joint sections were stained with H&E, and synovial thickness was measured as described in Materials and Methods using Bioquant Nova software. Data are means ± SEM of four (poly(IC), IFN-α) and six (arthritis and control) mice in each treatment group and represent two independent experiments. Differences relative to positive control arthritic mice were significant as shown: *, p < 0.01; ***, p < 0.001. B, Proliferation of synovial cells. C57BL/6 mice were injected with anti-collagen Ab and LPS and treated with IFN-α and poly(IC) as before and sacrificed on day 6; synovial tissue sections from hind paws were stained with anti-Ki67 Ab and counterstained with Contrast GREEN. Nonproliferating cells stain light bluish green, whereas proliferating cells stain dark blue/purple. C, Synovial tissue sections from B were analyzed using Bioquant Nova software and the percent of synovial cells positive for nuclear Ki67 is shown. Significant differences relative to positive control arthritic mice are marked: *, p < 0.05.
part, by suppressing synoviocyte proliferative responses to inflammation. This possibility was further investigated using immunohistochemistry with a mAb against Ki67 to detect proliferating cells (Fig. 5, B and C). There was a substantial increase in cells that stained positive for Ki67 (cells that stain dark blue/purple in Fig. 5B), indicating increased synovial cell proliferation in arthritic relative to control joints, (Fig. 5B, quantitated in Fig. 5C). Both IFN-α and poly(IC) suppressed synovial cell proliferation (Fig. 5, B and C), and this difference was statistically significant (Fig. 5C).

In addition, the number of newly formed blood vessels (positive for CD31) was diminished in IFN-α- and poly(IC)-treated animals, but this trend did not reach statistical significance (data not shown). Finally, we measured the effects of IFN-α and poly(IC) on synovial inflammatory mediators. The CAIA model is highly dependent on IL-1 (28, 31), and both IFN-α and poly(IC) suppressed synovial expression of IL-1 mRNA (Fig. 6A). In addition, IFN-α and poly(IC) suppressed synovial levels of cyclooxygenase-2 and VEGF (Fig. 6A). In contrast, synovial expression of RANTES and MCP-1 was not decreased by IFNα and poly(IC) (Fig. 6A). Synovial IL-10 expression was not affected by poly(IC) (data not shown), suggesting that systemic poly(IC) administration is insufficient to induce synovial IL-10 production. LPS contributes to inflammatory cytokine production in the CAIA model. We tested whether poly(IC) can suppress the inflammatory response to LPS.

Macrophages preincubated with poly(IC) for 24 h showed substantially reduced expression of IL-6, IL-1, and TNF-α after stimulation with LPS (Fig. 6B), a suppressive effect that is most likely mediated by type I IFN (22). Taken together, the results suggest that IFN-α and poly(IC) inhibit arthritis by suppressing synovial production of inflammatory mediators and synoviocyte proliferative responses.

**Discussion**

A role for TLRs in the induction and perpetuation of autoimmune and inflammatory diseases has been established (2, 5, 37). The major finding of our study is that, in contrast to other TLRs, activation of TLR3 suppresses the inflammatory effector phase of arthritis. TLR3 is a relatively weak inducer of inflammatory cytokines such as TNF and IL-1 and a strong inducer of type I IFN production. Our study suggests that this pattern of TLR3-induced cytokine production can attenuate inflammatory processes and implicate TLR3 as a potential negative regulator of inflammatory diseases via production of type I IFNs.

Recent reports showing that TLR9 suppresses intestinal inflammation via production of IFNs (20), and that TLR9 suppresses inflammation in the MRL/lpr model of systemic lupus erythematosus by an as yet unknown mechanism (38). Taken together, our results
and these previous reports suggest a regulatory function for certain TLRs in inflammatory processes.

TLR3 is expressed on the plasma membrane and in endosomes and recognizes extracellular and endocytosed dsRNA. Thus, TLR3 likely mediates the systemic effects of poly(IC) after injection of mice. In contrast, the RNA helicases RIG-I and Mda5 recognize cytoplasmic dsRNA, and transfection of cells with poly(IC) is required to activate these receptors (3). However, a recent publication suggests a role for Mda5 in mediating effects of poly(IC) in vivo (39), and future work will determine the relative importance of TLR3, RIG-I, and Mda5 in mediating the suppressive effects of dsRNA on inflammatory arthritis. TLR3, RIG-I, and Mda5 all participate in innate responses to viral infection and activate similar downstream signaling pathways, with sequential activation of TNFR-associated factor-associated NF-κB activator-binding kinase/1κB kinase-ε and IFN regulatory factor 3 resulting in production of large amounts of type I IFN.

Many factors have opposing effects on arthritis depending on whether they are expressed systemically or locally in the joint. For example, systemic TGF-β administration suppresses arthritis by suppressing immune and inflammatory reactions (40), whereas the chemotactic properties of TGF-β predominate after intra-articular injection, resulting in influx of inflammatory cells and induction of arthritis (41). Similarly, many TLR ligands, including TLR3 and TLR9 ligands, induce local cytokine and chemokine production and arthritis when injected intra-articularly (5, 23, 24). Articular ligation of these TLRs, such as occurs during joint infections, will contribute to synovial inflammation. Ligands of at least two TLRs, TLR3 and TLR9 (D. Mathis, unpublished observation), suppress arthritis when given systemically. Systemic ligation of these TLRs, which can occur during systemic infection, release of endogenous nucleic acids from apoptotic or necrotic cells, or therapeutic administration of TLR ligands, will suppress synovitis.

Type I IFNs can either exacerbate or attenuate autoimmune diseases. For example, type I IFNs have been implicated in the pathogenesis of the autoimmune disease systemic lupus erythematosus but have clear suppressive and therapeutic effects in multiple sclerosis and inflammatory bowel disease (17–21). Greater understanding of which components of these complex diseases are expressed by IFNs and of the cellular and molecular basis of the beneficial effects of type I IFNs would help guide future therapeutic approaches. This study shows that type I IFNs suppress the inflammatory effector component of arthritis by a mechanism that involves inhibition of synovial cell proliferation and of inflammatory cytokine production. Although type I IFNs suppressed arthritis in other experimental models, a significant clinical benefit was not detected in relatively short term trials of IFN in RA (42–48). We predict that prolonged IFN therapy that suppresses the expansion of pannus by inhibiting the growth of synoviocytes would potentially have a beneficial effect on inflammatory arthritis.

In contrast to suppressing inflammation, type I IFNs exacerbate autoimmunity (15, 16). We propose that the balance between the opposing effects of IFNs on inflammation and autoimmunity will determine the outcome of IFN treatment of specific diseases. In RA, this balance would be determined by the opposing effects of IFN on promoting autoimmunity mediated by lymphocytes and dendritic cells and suppressive effects on inflammation that is mediated by synovial macrophages and fibroblasts. Autoimmunity has been proposed to play a more important role in pathogenesis in early RA, with inflammatory synovitis becoming less dependent on autoimmunity later in the disease process (49). If this notion is correct, our results showing effective suppression of the inflammatory synovial component of arthritis by IFN-α suggests that IFN therapy may be more effective in late disease, especially if therapy is of sufficient duration to attenuate the expansion of pannus. On the other hand, IFNs would be least effective and could potentially even exacerbate disease, if given during a phase of arthritis where autoimmunity is a prominent driver of synovial inflammation.

Macrophages play an important role in the pathogenesis of multiple sclerosis and inflammatory bowel disease, and one mechanism by which IFNs suppress these diseases is inhibition of macrophage inflammatory cytokine production (19–21). Macrophages also play a key role in the pathogenesis of RA and of the effector inflammatory phase of experimental arthritis (50). Thus, it is likely that suppression of macrophage cytokine production contributes to the suppressive effects of IFNs that we observed. This notion is supported by our findings of lower expression of the macrophage-derived cytokine IL-1 in synovia of IFN-α or poly(IC)-treated arthritic mice (Fig. 6A), and of a suppressive role of poly(IC) in proinflammatory cytokine production by macrophages in vitro (Fig. 6B). Emerging evidence suggests that type I IFNs also suppress inflammatory diseases by direct effects on target organs or tissues, for example by regulating epithelial homeostasis in the bowel (51). It is likely that type I IFNs suppressed synovitis at least in part by acting directly on cells in the target tissue, namely by suppressing synoviocyte proliferation (Fig. 5) via their well-known effects of suppressing progression through the cell cycle (13).

Synovial biopsies have demonstrated that RA patients can have substantial inflammatory infiltrates in synovium obtained from asymptomatic joints that have a normal physical examination (52–54). This work, together with evidence of autoimmunity against cyclic citrullinated peptide before onset of clinical disease (55, 56), demonstrate a preclinical phase of disease where joint swelling is not apparent, despite intense synovial inflammation. These previous findings suggest that even intense synovial inflammation may be clinically unapparent in the absence of a quantitative increase in the size of infiltrates (as occurs during pannus formation) or the development of vascular permeability that leads to joint effusions. We found that even when IFN and poly(IC) effectively suppressed joint swelling (Figs. 1 and 3 and Table I), inflammatory synovial infiltrates, deposits of fibrin in the joint cavity, and loss of proteoglycans were readily detectable. This pattern is similar to that of biopsied asymptomatic joints of RA patients and suggests that type I IFNs suppressed the progression of synovial inflammation into clinically apparent disease. Our results suggest that IFNs accomplished this suppression by inhibiting synoviocyte proliferation (Fig. 5) and thus pannus formation, by suppressing expression of regulators of increased vascular permeability such as cyclooxygenase-2 (via PGE2) and vascular endothelial growth factor (Fig. 6A), and possibly by suppressing neoangiogenesis (A. Yarilina, unpublished observations). Thus, type I IFNs can suppress progression of synovitis by several mechanisms that include suppression of proliferation and production of inflammatory factors, and possibly by modulation of factors that regulate synovial vasculature.

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Disclosures
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References


