Endothelial Cells and Fibroblasts Amplify the Arthritogenic Type I IFN Response in Murine Lyme Disease and Are Major Sources of Chemokines in *Borrelia burgdorferi*-Infected Joint Tissue

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Endothelial Cells and Fibroblasts Amplify the Arthritogenic Type I IFN Response in Murine Lyme Disease and Are Major Sources of Chemokines in *Borrelia burgdorferi*-Infected Joint Tissue

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Localized elevation in type I IFN has been uniquely linked to the severe Lyme arthritis that develops in C3H mice infected with the spirochete *Borrelia burgdorferi*. In this study, the dynamic interactions that result in generation of these responses were further examined in C3H mice carrying the type I IFN receptor gene ablation, which effectively blocks all autocrine/paracrine signaling crucial to induction of downstream effectors. Reciprocal radiation chimeras between C3H and IFNAR1−/− mice implicated both radiation-sensitive and radiation-resistant cells of the joint tissue in the proarthritic induction of type I IFN. Ex vivo analysis of cells from the naive joint revealed CD45+ cells residing in the tissue to be uniquely capable of initiating the type I IFN response to *B. burgdorferi*. Type I IFN responses were analyzed in real time by lineage sorting of cells from infected joint tissue. This demonstrated that myeloid cells, endothelial cells, and fibroblasts were responsible for propagating the robust IFN response, which peaked at day 7 postinfection and rapidly resolved. Endothelial cells and fibroblasts were the dominant sources of IFN signature transcripts in the joint tissue. Fibroblasts were also the major early source of chemokines associated with polymophonuclear leukocyte and monocyte/macrophage infiltration, thus providing a focal point for arthritis development. These findings suggest joint-localized interactions among related and unrelated stromal, endothelial, and myeloid cell lineages that may be broadly applicable to understanding the pathogenesis of diseases associated with type I IFN signature, including systemic lupus erythematosus and some rheumatoid arthritides. *The Journal of Immunology*, 2012, 189: 000–000.

Lyme disease in humans is caused by infection with the tick-borne spirochete *Borrelia burgdorferi* and results in clinical arthritis in up to 30% of infected individuals (1, 2). Lyme arthritis has been extensively studied in the C3H mouse, which replicates many of the features of acute human disease, including edema, synovial hyperplasia, inflammatory cell infiltration, and reactive/reactive changes associated with joint tissue (3). A range of arthritis severity has been observed in humans and in different inbred strains of mice, as initially reported by Steere and Barthold (3). Previous global gene expression analysis in the joint tissue of C3H mice revealed an early inflammatory response at 1 wk of infection, weeks prior to the development of arthritic lesions (9). This early transcriptional event was characterized by robust but transient induction of IFN-responsive transcripts, and was absent from the mildly arthritic B6 mice. Innate immune production of type I IFNs (IFN-α,β) was suspected, as type II IFN (IFN-γ) is not required for Lyme arthritis development in C3H mice (10). Additionally, the peak of IFN-inducible transcript induction was prior to infiltration of lymphocytes into joint tissue likely to be required for IFN-γ production (11, 12). The involvement of type I IFN in Lyme arthritis was subsequently confirmed through the systemic administration of a type I IFN receptor (IFNAR1)-blocking mAb that was capable of disrupting signaling by all type I IFNs. This treatment suppressed the spike in IFN-inducible transcripts in the joint tissue at 1 wk of infection and the subsequent development of arthritis at 4 wk postinfection (13). In contrast, blocking IFN-γ suppressed expression of many of the overlapping IFN-inducible transcripts, but did not result in reduced arthritis severity.

The unique contribution of type I IFN to the development of severe Lyme arthritis in C3H mice implies specialized targets for this IFN in the infected joint tissue that cannot be compensated with IFN-γ. The potential importance of this finding is underscored by the pathological role of type I IFNs in systemic lupus erythematosus (SLE) and in the injurious side effects associated with IFN-αβ–based therapies for multiple sclerosis and hepatitis C.
infection (14–16). Even more relevant to Lyme arthritis pathogenesis are recent studies implicating type I IFN in a subgroup of rheumatoid arthritis (RA) patients who fail to respond to therapeutic TNF blockade (17–19). Thus, studies with Lyme arthritis may broadly improve our understanding of immune-mediated inflammatory diseases by providing insight for patient groups currently not well served by existing therapies.

To further our understanding of the contribution of type I IFN signaling in the development of Lyme arthritis, the IFN receptor 1 gene ablation (IFNAR1−/−) was crossed onto the C3H background (C3H IFNAR1−/−). Arthritis severity was reduced in the absence of IFNAR1. The development of radiation chimeras between C3H and IFNAR1−/− mice allowed assessment of contributions of both myeloid lineage and parenchymal cells to the proarthritis IFN response: both developmental lineages were involved. Ex vivo recovery of sorted cells from the joint tissue revealed dynamic contributions of various cell lineages to the arthritis-promoting IFN response. Resident myeloid cells of the joint tissue were identified as the initiators of type I IFN production upon encounter with B. burgdorferi, whereas endothelial cells and joint fibroblasts expressing adhesion/activation endothelial markers were found to amplify the response and served as the major source of disease-promoting chemokines. The development of severe arthritis was determined to be orchestrated by a cascade of events initiated by interaction of B. burgdorferi with myeloid, stromal, and endothelial cells at 1 wk postinfection.

**Materials and Methods**

**Mice, bacterial cultures and infections, and assessment of arthritis severity**

C3H/HeN mice were obtained from Charles River Breeding Laboratories or from National Cancer Institute, and C57BL/6 mice were from National Cancer Institute. The IFNAR1 gene ablation from the C57BL/6 mouse (20) (provided by M.-K. Kaja, University of Washington, Seattle, WA) was crossed six generations onto the C3H background. Filial mating was performed to generate C3H/HeN IFNAR1−/−. All mice were housed in the University of Utah Animal Research Center (Salt Lake City, UT) following all institutional guidelines for the care and use of mice in biomedical research. Mice were infected with 2 × 10^9 bacteria of the clonal B. burgdorferi strain N40 by intradermal injection into the skin of the back (3). Infected and control C57BL/6 mice received 5 × 10^4 U universal type I IFN (PBL) on day 1 and 10^4 U every other day for 28 d by i.p. injection, or infected and control C57BL/6 mice received 5 × 10^4 bacteria of the clonal B. burgdorferi strain N40 by intradermal injection into the skin of the back (3).

**Preparation of single-cell suspensions from mouse tissue**

Single-cell suspensions were prepared from the rear ankle joint tissue, following removal of skin. Joint tissue was partially removed from bone using 20-gauge syringe needles to facilitate digestion by incubation in RPMI 1640 containing 0.2 mg/ml endotoxin-free Liberase TM (Roche) and 100 μg/ml DNase I (Sigma-Aldrich) for 1 h at 37°C. After incubation, gentle pipetting further disrupted tissue and intact tissue was broken apart using the end of a 5-ml syringe. The single-cell suspension was filtered through a 100-μm cell strainer and centrifuged, and the RBCs were lysed using ammonium-chloride-potassium lysing buffer. Blood was collected in Eppendorf tubes containing acid citrate dextrose, and leukocyte populations were analyzed, as described (23).

**Flow cytometry**

All flow cytometry data were analyzed using BD CellQuest Pro software. Sorting experiments were performed using a BD FACSaria II. All other FACS data were collected on a BD FACS Canto II flow cytometer or BD LSRII flow cytometer. The 7-amo-inoctinomycin D (eBioscience) or DAPI (Invitrogen) was used in all experiments, and dead cells were excluded from analyses, as were doublet cells. All Abs used for flow cytometry were purchased from either BioLegend or eBioscience. Unconjugated Fc, blocking Ab (clone 93; BioLegend) was included in all Ab-labeling experiments. Position of gates for sorting and analysis was based on analysis of appropriate isotype controls. Fluorochrome-conjugated Abs and isotype controls used in this study were as follows: FITC-conjugated anti-CD11b (M1/70) and anti-B220 (RA3-6B2); PerCP/Cy5.5-conjugated anti-Ly6c (HK1.4) and anti-CD31 (390); PE-conjugated anti-CD45 (YN1/1.7.4), anti-CD31 (390), anti-IFNAR1 (MAR1-S33), and anti-CD29 (HMβ1-1); PE/Cy-7-conjugated anti-CD11b (M1/70), anti-CD90.2 (30-H12), and anti-CD45.2 (104); allophycocyanin-conjugated anti-F4/80 (BAM8), anti-TCR β (H5.97.57), anti-CD29 (HMβ1-1), anti-CD45 (30-F11), and anti-CD16 (104/292); Alexa Fluor 700-conjugated anti-Ly6G/Ly6C (RB6-8C5) and anti-CD45.2 (104); Pacific Blue-conjugated anti-TCR β (H5.97-57) and anti-B220 (RA3-6B2); and biotin-conjugated anti-PE (PK001) and PE-conjugated streptavidin.

**Injection of mAbs**

The following Abs were used in vivo for cytokine neutralization: anti–IFN-γ (XMGl.2), anti–TNF-α (XT3.11), and rat IgG1 (HRP) isotype control, and were aggregate, endotoxin free, and sterile (Bio X Cell). Groups of five to six mice received 1 mg indicated Ab 1 d prior to infection, followed by 0.5 mg of the same Ab every 4–5 d thereafter, all by i.p. injection (11, 13).

**Isolation of RNA and quantitative RT-PCR**

For all experiments examining gene expression in joint tissue, mice were excised, frozen immediately in dry ice/ethanol, and stored at −80°C. Total RNA from joint tissue and cultured cells was performed using TRIzol reagent (Invitrogen) (24). RNA from FACS-sorted cell populations was purified with the RNAeasy kit (Qiagen). RNA recovered from tissue and cells was reverse transcribed, and transcripts were quantified using a 7900HT Fast Real-Time PCR System (Applied Biosystems) and the 2^(-ΔΔCT) method. For real-time PCR, primer pairs were designed from the complete genomic sequence of B. burgdorferi stains N40 and B31 (25). Primer sequences used in this study were as follows: 5'-CCCCATGTTAGCGGTGAGACGAGC-3', reverse 5'-GGTTGGTGGTGGAAAAGTATGAGG-3'; Ccl2 forward 5'-GCTTTTTGTGTCGCTGATAG-3', reverse 5'-CATTGTGTTTACATCCTCCTG-3'; Cxcl1 forward 5'-ATGGCCTGGATTTACCTCCTC-3', reverse 5'-CTTCGACCCCAAGCAAG-3'; Cxcl2 forward 5'-CTCCCTTCCCATTTCCGCCGAG-3', reverse 5'-AGGTGCTAGTGTGAGCCGCTT-3'; Fasl forward 5'-GCAGTGTTGATCTTCCAGTGGG-3', reverse 5'-TCTCCCTTTCTCTCCGGGAG-3'; Icam1 forward 5'-CD45 forward 5'-AGGGCTGTAGATTGTCCTCTA-3', reverse 5'-CTTCAGAACGAGGAAACACG-3'; Pecam1 (CD31) forward 5'-CTCCCTCACATCAACAGCACC-3', reverse 5'-TTTGTGCAGCTGTCATTGCTGCG-3'; Pip γ (CD45) forward 5'-GCTTCACCATACAATGAGGCG-3', reverse 5'-TGGTGGCCTGTTTCCTTC-3'; Thxl (CD90) forward 5'-GGATGAGGGGCGGACCTTGGT-3', reverse 5'-CTTCTGATTCTGCACTG-3'; Vcam1 (CD106) forward 5'-CCTGGACTGATGCTTGGACTG-3', reverse 5'-GGTCAGTGGGAGACCAAGC-3'. Primer sequences for β-actin, Igk, Igλ, Mmp3, Stat1, Cxcl13 (9), Cxcl9, Cxcl10, Oasl2 (13), Ifi, Gbp2 (25), Toxa, and Ifnβ (26) can be found in the indicated citations.

**Isolation of DNA and quantification of joint spirochetes**

For quantification of joint spirochetes at 4 wk postinfection, total DNA was isolated from joint tissue, as described. PCR quantification of spirochetes was performed by amplification of the B. burgdorferi recA gene and normalized to the mouse Nido gene using a Roche LC-480 (27).

**Generation of reciprocal radiation chimeras**

The diminished severity of Lyme arthritis in mice 10 wk and older required the development of a protocol allowing rapid reconstitution of irradiated mice with high numbers of hematopoietic cells (11). C3H and C3H IFNAR1−/− littermate mice of age were lethally irradiated with 2 doses of 525 Gy 3 h apart using a GE Isovolt Titan. Twenty-four hours following irradiation, splenocytes were harvested from C3H or C3H IFNAR1−/− donor mice, and 2 × 10^7 splenocytes in a 200 μl volume were injected i.v.
into each irradiated recipient. Chimerism was determined at 6 wk postirradiation by flow cytometry assessment of mAb anti-IFNAR1 expression by blood leukocytes (28). Staining required sequential treatment with PE-conjugated anti-IFNAR1, biotin-conjugated anti-PE, and PE-conjugated streptavidin, which allowed sufficient fluorescence intensity to readily distinguish wild-type from IFNAR1−/− cells. Peripheral blood B cells and monocytes were found to be >90% donor derived, whereas T cells were ~60% donor derived. Total blood leukocyte counts were comparable to those from nonirradiated control mice 7 wk posttransplantation. Mice were infected at 3 wk postirradiation and transplantation, 7–8 wk of age and, therefore, allowing Lyme arthritis to be assessed.

**Cell culture**

Bone marrow-derived macrophages (BMDM) were isolated from the femurs and tibias of mice, as previously described (29). Macrophage cultures were plated in 12-well dishes at a density of 6 × 10⁷/ml in media containing the serum replacement Nutridoma (Roche) and stimulated with live *B. burgdorferi* cn40 (7.4 × 10⁹/ml), 10 U/ml IFN-γ (Sigma-Aldrich), or PBS. Macrophage cultures were stimulated at 37°C, 5% CO₂, and harvested either at 6 h for RNA extraction or at 24 h for assessment of type I IFN in supernatants by bioassay.

**Magnetic separation of leukocytes and stromal cells from naive joint tissue**

Single-cell suspensions of joint tissue were labeled with biotinylated anti-CD45.2 (BioLegend), followed by labeling with streptavidin magnetic beads (Miltenyi Biotec), and magnetic separation was performed according to the manufacturer’s instructions, with sequential application to a second column. Relative purity of the CD45.2⁺ and CD45.2⁻ populations was determined by flow cytometry using allophycocyanin-conjugated anti-CD45 (clone 30-F11), which recognizes an epitope distinct from the Ab used in magnetic bead sorting (anti-CD45.2, clone 104). Unfractionated and (clone 30-F11), which recognizes an epitope distinct from the Ab used in magnetic bead sorting (anti-CD45.2, clone 104). Unfractionated and (clone 30-F11), which recognizes an epitope distinct from the Ab used in magnetic bead sorting (anti-CD45.2, clone 104). Unfractionated and

**Type I IFN bioassay**

Bioactive type I IFN was detected in culture supernatants from BMDM incubated for 24 h with *B. burgdorferi* in the presence or absence of IFN-γ (eBioscience) by bioassay with B16-Blue IFN-α cells (InvivoGen), following manufacturer’s directions. Standard curve was generated with mouse rIFN-β (PBL).

**Data and statistical analyses**

All graphical data represent the mean ± SEM. Statistical analysis was performed using Prism 5.0c software. Multiple-sample data sets were analyzed by one-way ANOVA with appropriate post hoc test for pairwise comparisons (Figs. 2–6, Tables II, III). Two-sample data sets were analyzed by Student’s *t* test (Figs. 1, 8, Table I). Categorical data for histopathology was assessed by the Mann–Whitney *U* test (Figs. 1, 3, Table I). Statistical significance (*p < 0.05*) is indicated by *.

**Results**

Lyme arthritis severity can be modulated by augmentation or ablation of type I IFN signaling

We previously demonstrated that administration of a blocking mAb to the type I IFN receptor resulted in a significant diminution of arthritis severity in *B. burgdorferi*-infected C3H mice, implicating the type I IFN autocrine/paracrine pathway in arthritis development (13). As this pathway is not upregulated in the joint tissue of arthritis-resistant B6 mice, we tested the effect of supplementation of *B. burgdorferi*-infected B6 mice with type I IFN throughout *B. burgdorferi* infection. Treatment of B6 mice with daily injections of IFN-α for 4 wk following infection resulted in significantly greater ankle swelling than observed in the control group, receiving daily injections of PBS (Table I). Histopathologically assessed lesion scores suggested a trend toward increased arthritis in the group receiving IFN-α; however, this did not achieve statistical significance. Importantly, the increase in ankle swelling in treated B6 mice (Table I) did not reach the degree of severe arthritis seen in the genetically susceptible C3H mice (Fig. 1). This finding may further indicate the presence of IFN regulatory mechanisms inherent to the B6, but not the C3H genetic background.

As the previous assessment of type I IFN in Lyme arthritis was performed in vivo using a mAb to prevent signaling though the cognate receptor, a more rigorous approach was used by crossing the IFNAR1 gene disruption onto the susceptible C3H genetic background. Marker-assisted protocols were employed for rapid and complete crossing and to ensure that none of the quantitative trait loci associated with Lyme arthritis severity were lost from the recipient C3H mice (30). Infection of C3H IFNAR1−/− mice with *B. burgdorferi* revealed a significant reduction in arthritis severity relative to wild-type C3H mice, as demonstrated by the traits of ankle swelling, overall lesion score, and neutrophil infiltration (Fig. 1), with less robust differences in tendon sheath thickness and reactive/reparative abnormalities also observed (data not shown). These results mirror the significant but incomplete reduction in arthritis severity previously reported in C3H mice treated with IFNAR receptor-blocking Ab prior to infection, thus indicating that the observed partial reduction in arthritis was not reflective of incomplete neutralization by the Ab (13). Three lines of experimental evidence now support the unique involvement of type I IFN in arthritis development in C3H mice, as follows: 1) arthritis can be partially suppressed with receptor-blocking mAb; 2) arthritis is similarly reduced by genetic ablation of the IFN signaling pathway; and 3) ankle swelling can be partially restored in B6 mice by administration of exogenous IFN-α. A modest increase in *B. burgdorferi* levels in joint tissue of IFNAR1−/− mice was observed, and demonstrated that the decreased arthritis seen in the mutant mice was not secondary to reduced numbers of spirochetes in the tissue (Fig. 1).

<table>
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<th>Table I. Effect of IFN-α administration on arthritis development in <em>B. burgdorferi</em>-infected B6 mice</th>
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<td>Infection Status</td>
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Assessed at 4 wk of infection.

*Values represent mean ± SD.

*Statistical significance between PBS- and IFN-α-treated group, *p < 0.05.*
dorferi-infected C3H IFNAR1−/− mice, shown for Cxcl10, Oasl2, Igtp, and Gbp2 in Fig. 2A, although at much lower levels than previously reported in wild-type C3H mice (9, 13, 24).

Recent reports in other infection models have identified modifying effects of type I IFN on IFN-γ production (31, 32) as well as MyD88-dependent modulation of IFN-β production (33). The identification of low levels of transcripts for both IFN-γ and TNF-α in the joint tissue of infected C3H IFNAR1−/− mice (data not shown) suggested that one or both might be responsible for induction of the IFN profile in absence of type I IFN signaling. Injecting infected C3H IFNAR1−/− mice with neutralizing Abs for IFN-γ or TNF-α allowed testing of this possibility. Treatment with anti-TNF resulted in detectable, but not significant, reduction in the expression of several IFN-inducible transcripts in infected joint tissue when compared with mice treated with an isotype control mAb (Fig. 2A). In contrast, treatment with IFN-γ neutralizing mAb resulted in complete suppression of the expression of IFN-inducible genes to levels found in uninfected mice (Fig. 2A). Thus, the residual profile of IFN-inducible transcripts in infected IFNAR1−/− mice can be attributed to compensatory effects of IFN-γ, possibly reflecting an enhanced effect permitted by the absence of type I IFN modulatory activity.

IFN-γ partially compensates for type I IFN signaling ablation in the BMDM response to B. burgdorferi, but does not substitute for type I IFN in arthritis development

The B. burgdorferi-induced upregulation of IFN-inducible transcripts in BMDM was previously shown to be dependent on IFN receptor-mediated autocrine/paracrine signaling in B6 mice (13). BMDM were prepared from C3H and C3H IFNAR1−/− mice, and the presence of the receptor was again shown to be necessary for upregulation of IFN-inducible transcripts in response to B. burgdorferi, shown for Cxcl10, Oasl2, Igtp, and Gbp2 (Fig. 2B). Importantly, C3H IFNAR1−/− BMDM were able to respond to B. burgdorferi by other sensing/signaling pathways, as indicated by upregulation of Tnfa transcripts at concentrations similar to C3H BMDM (Fig. 2C).

The mAb results in Fig. 2A suggested that IFN-γ might compensate for type I IFN signaling in induction of the IFN transcriptional response to B. burgdorferi. To model the potential of IFN-γ to compensate for type I IFN within the joint, exogenous IFN-γ was added to BMDM cultures of C3H and C3H IFNAR1−/− macrophages stimulated with B. burgdorferi (Fig. 2B). Treatment with rIFN-γ alone resulted in the induction of most transcripts in both wild-type and IFNAR1−/− macrophages, but with a range of expression, shown for Cxcl10, Oasl2, Igtp, and Gbp2. Costimulation with IFN-γ and B. burgdorferi resulted in expression of IFN-inducible transcripts in both C3H and C3H IFNAR1−/− macrophages, shown for Cxcl10, Igtp, and Gbp2 in Fig. 2B. However, expression of Oasl2, a transcript linked to early type I IFN responses, was not upregulated in C3H IFNAR1−/− macrophages costimulated with IFN-γ and B. burgdorferi. The reduced expression of Igtp and Gbp2 when stimulated simultaneously with IFN-γ and B. burgdorferi may reflect a rapid response to dual stimuli that was missed by the 6-h time point (Fig. 2B).

Interestingly, transcriptional induction of IFN-β was observed at low concentrations in response to B. burgdorferi in both wild-type and IFNAR1−/− macrophages and was further upregulated by the addition of IFN-γ (Fig. 2C). The induction of IFN-β transcripts in C3H IFNAR1−/− macrophages defines this early production (6 h) as independent of positive feedback through type I IFN receptors. To ensure that these transcripts reflected the translation and release of type I IFN protein (IFN-α and IFN-β), macrophage supernatants collected at 24 h were subjected to bioassay for type I IFN using the B16-Blue cell line (InvivoGen) (Fig. 2C). Type I IFN secretion was detected in C3H BMMDM stimulated with B. burgdorferi, whereas IFN-γ alone did not have this effect. Treatment with IFN-γ enhanced production of type I IFN protein in responses to B. burgdorferi, in macrophages from both C3H and C3H IFNAR1−/− mice. Of note, bioassay results were confirmed to be specific for type I IFN as these findings were not influenced by the addition of neutralizing Ab to IFN-γ (data not shown).

The observation that the residual IFN profile seen in infected C3H IFNAR1−/− was suppressed by anti–IFN-γ neutralizing mAb (Fig. 2A) clearly implicates IFN-γ in the localized response to B. burgdorferi in the joint of C3H IFNAR1−/− mice, and suggests it could contribute to the residual arthritis seen in these mice. To test this hypothesis, groups of five infected C3H IFNAR1−/− mice were administered IFN-γ neutralizing mAb or isotype control mAb by i.p. injection every 5 d for 4 wk. IFN-γ neutralization did not cause further reduction in the severity of arthritis in C3H IFNAR1−/− mice: average ankle swelling for five mice treated with isotype control was 0.80 ± 0.23 mm, whereas mice treated with anti–IFN-γ measured 0.803 ± 0.17 mm. This finding indicates that the IFN-γ-dependent induction of transcripts in the joint tissue of infected IFNAR1−/− mice at 1 wk postinfection does not contribute to the residual arthritis seen at 4 wk postinfection, consistent with our previous published results employing blocking mAbs in C3H mice (13). Therefore, the residual arthritis seen in B. burgdorferi-infected C3H IFNAR1−/− mice develops independently of either type I or type II IFN.
Relative contribution of radiation-sensitive and resistant cells to the type I IFN-dependent development of Lyme arthritis

The results of Fig. 2 suggest that a mixture of cell lineages in the joint tissue may determine both the magnitude and breadth of the IFN response to B. burgdorferi and the severity of subsequent arthritis in C3H mice. To address the relative contribution of resident cells of the joint, such as endothelial cells and fibroblasts, and infiltrating hematopoietic cells to the type I IFN-dependent development of arthritis, we developed reciprocal radiation chimeras between C3H and C3H IFNAR1^{-/-} mice, using rapid reconstitution protocol to allow B. burgdorferi infection of mice, 8 wk of age. The efficiency of reconstitution of hematopoietic cells in the chimeras was determined by staining for IFNAR1 (28), described in Materials and Methods. Reconstitution of irradiated C3H mice with syngeneic splenocytes (C3H⇒C3H) resulted in severe arthritis following infection by B. burgdorferi, whereas infection of irradiated C3H IFNAR1^{-/-} mice reconstituted with syngeneic splenocytes (IFNAR1^{-/-}⇒IFNAR1^{-/-}) displayed less severe arthritis (Fig. 3), and similar to nonirradiated mice in Fig. 1. Reconstitution of C3H mice with splenocytes from IFNAR1^{-/-} mice or reconstitution of IFNAR1^{-/-} mice with C3H splenocytes resulted in arthritis of intermediate severity following infection, shown for joint measurement and overall lesion score (Fig. 3). Control of B. burgdorferi was not significantly different in the treated animals, demonstrating that reconstitution was adequate for host defense (Fig. 3). That arthritis severity in the chimeras was intermediate compared with that observed for wild-type or IFNAR1^{-/-} mice implies that both radiation-resistant cells of the joint and radiation-sensitive hematopoietic cells contribute to the IFN receptor-dependent autocrine/paracrine effect that drives the severe arthritis of C3H mice.

Ex vivo identification of cell lineages in the joint tissue of naive mice capable of initiating and responding to the type I IFN response

The radiation chimera experiment of Fig. 3 implicated both hematopoietic and resident cells of the joint in the type I IFN-
myeloid cells, such as macrophages, are uniquely endowed with the ability to internalize and sense *B. burgdorferi* pathogen-associated molecular patterns, which lead to the initiation of the IFN-responsive transcriptional profile (36–38). To identify the relative ability of hematopoietic cells and nonhematopoietic cells of the joint to initiate the IFN profile, single-cell suspensions were recovered from the joints of naive C3H mice following gentle digestion, and fractionated into CD45+ and CD45− populations by magnetic bead separation. Approximately 20% of the cells in the unfractionated group were CD45+, and this increased to 75–80% following CD45 enrichment, as determined by flow cytometry (Fig. 4A). Cells from the three populations were cultured for 6 h in the presence of B. burgdorferi, IFN-β, or B. burgdorferi plus IFN-β. The expression of the NF-κB–dependent transcript *Tnfa* served as a control for viability, as its production has been previously characterized in both myeloid and endothelial cells treated with *B. burgdorferi* (22, 39). By this measure, both CD45+ and CD45− fractions were viable and capable of responding to *B. burgdorferi* (Fig. 4B). Cell viability was further confirmed by the response to IFN-β alone, as both CD45+ and CD45− fractions upregulated the early IFN-inducible transcripts *Cxcl10* and *Tyki* following this treatment (Fig. 4C, 4D) (40). This also points to the potential involvement of both fractions in the amplification stage of the IFN response. In contrast, only the CD45+ cells were capable of up-regulating *Cxcl10* and *Tyki* in response to *B. burgdorferi* alone (Fig. 4C, 4D), indicating novel contribution of CD45+ cells in triggering the IFN-inducible profile. As we previously demonstrated that the IFN profile at 1 wk postinfection is also observed in infected C3H *scid* mice (13), these results strongly implicate a myeloid lineage cell as initiator of the IFN response resulting in activation of large numbers of resident cells that amplify the response.

**Assessment of changes in cellular composition and activation in *B. burgdorferi*-infected joint tissue**

The unique ability of CD45+ cells to initiate the IFN profile in vivo, and the ready induction of this response in BMDM cultures, suggested that infiltrating myeloid cells might be the driving force behind the IFN response to *B. burgdorferi* in vivo.

Ly6C+–expressing inflammatory monocytes have recently been implicated in both the beneficial type I IFN response to viral infection and in its pathological production in chronic disease such as SLE (41, 42). Therefore, the composition and infiltration of Ly6C+ myeloid-lineage cells to the joint tissue of C3H mice were
determined by flow cytometry assessment of populations released from the joint tissue over time: PMN leukocytes were defined as GR1<sup>high</sup>Ly6C<sup>dim</sup>CD11b<sup>high</sup>CD45<sup>+</sup>, macrophages were GR1<sup>−</sup>Ly6C<sup>−</sup>F4/80<sup>−</sup>CD11b<sup>high</sup>CD45<sup>+</sup>, and inflammatory monocytes were GR1<sup>dim</sup>Ly6ChighCD11b<sup>high</sup>CD45<sup>+</sup>. Increases in all three myeloid lineage populations were seen at days 11 and 14 postinfection relative to uninfected mice, with macrophages and PMNs dominating the inflammatory cell infiltrate (Fig. 5A). The increases in these lineages at day 11 postinfection were similar between C3H and C3H IFNAR<sup>−/−</sup> mice, indicating the recruitment or expansion of these cells was not dependent on type I IFN signaling. Importantly, the Ly6C<sup>+</sup> population showing the greatest increase following infection in both C3H and C3H IFNAR<sup>−/−</sup> mice was the inflammatory monocyte; however, this increase was not observed until day 11 of infection and was still elevated at day 14. Of note, there was no increase in any of the myeloid cell populations at day 7 postinfection, the time point previously and in this study associated with the peak of the IFN response (Fig. 5A). This striking result indicated that the induction of the IFN profile was not dependent on recruitment of myeloid cells from the blood or other tissues; rather, it suggested that the initiator of the IFN response might be a myeloid cell endogenous to the joint tissue. This could include macrophages naturally present within the joint space or synoviocytes of the macrophage lineage.

That Ly6C<sup>+</sup> myeloid lineage cells contribute to the ultimate development of arthritis is clearly suggested by the dramatic increase in these populations by day 11 postinfection. These cells may also be important in host defense, as their presence at 14 d coincides with our previous identification of upregulation of transcripts associated with host defense at this time point (9). In support of this concept, there was a marked shift in the ratio of PMN to macrophage presence at day 14 postinfection of wild-type mice that did not occur in the absence of IFN signaling (Fig. 5A), consistent with published findings in other experimental models of the role of type I IFN in maturation of the myeloid cells in localized tissues (42). It is interesting to speculate on the possible contribution of this difference to the development of more severe arthritis in C3H mice than in C3H IFNAR<sup>−/−</sup> mice.

Changes in cellularity of resident cells of the joint tissue were also found, as shown for the increase in both endothelial cells and fibroblasts in joints of infected mice (Fig. 5B). Endothelial cell (CD45<sup>−</sup>CD31<sup>+</sup>) and fibroblast (CD45<sup>−</sup>CD31<sup>−</sup>CD90<sup>+</sup>CD29<sup>+</sup>) numbers were increased by days 11 and 14 postinfection in joints from both C3H and C3H IFNAR<sup>−/−</sup> mice (Fig. 5B). Increases in these populations were similar in the two mouse strains, indicating lack of dependence on type I IFN. The percentages of endothelial cells and joint fibroblasts that displayed activation markers (VCAM1<sup>+</sup>ICAM1<sup>high</sup>) were increased by day 11 postinfection, and continued to be elevated through day 14. The similarity in activation marker expression by endothelial cells and joint fibroblasts from C3H and C3H IFNAR<sup>−/−</sup> mice indicated that this event was also not dependent on type I signaling (Fig. 5B).

FIGURE 5. Infiltration and expansion of myeloid cells, endothelial cells, and fibroblasts in the joint tissue of B. burgdorferi-infected C3H mice. (A) Single-cell suspensions were analyzed for the presence of PMNs (GR1<sup>+L</sup>y6C<sup>−</sup>CD11b<sup>−</sup>CD45<sup>+</sup>), inflammatory monocytes (Ly6C<sup>+</sup>GR1<sup>−</sup>CD11b<sup>−</sup>CD45<sup>+</sup>), and macrophages (GR1<sup>−</sup>Ly6C<sup>−</sup>F4/80<sup>−</sup>CD11b<sup>−</sup>CD45<sup>+</sup>) by flow cytometry at days 0 (media), 7, 11, and 14 postinfection with B. burgdorferi. Mean ± SEM are indicated, with n = 3. (B) Single-cell suspensions were analyzed for fibroblasts (CD45<sup>−</sup>CD31<sup>−</sup>CD90<sup>−</sup>CD29<sup>+</sup>) or endothelial cells (CD45<sup>−</sup>CD31<sup>−</sup>) by flow cytometry at days 0 (media), 7, 11, and 14 of infection. Activated cells were identified as VCAM1<sup>+</sup>ICAM1<sup>high</sup>. Statistically significant differences were found at 11 and 14 d of infection relative to uninfected mice, for both the C3H and IFNAR<sup>−/−</sup> mice, but differences were not found between the two mouse genotypes at any time point (*p < 0.05) indicated. These results are representative of two separate experiments, n = 3.

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However, the potential participation of these cells in the amplification of the IFN response and participation in other proinflammatory cascades in C3H mice is clearly supported by the expression of classic activation markers. Thus, the complex environment of the infected joint tissue provides opportunity for activation of multiple cell types that contribute to the IFN-dependent and IFN-independent events associated with the development of Lyme arthritis.

Ex vivo analysis of IFN-responsive cells sorted from the joint tissue of B. burgdorferi-infected mice

The presence of activated endothelial cells and fibroblasts in the joint tissue of infected C3H mice suggested that they could be early participants in the response to B. burgdorferi, in addition to resident myeloid lineage cells (Figs. 4, 5). To quantitatively analyze the IFN response within the infected joint tissue, single-cell suspensions were prepared by enzymatic digestion, stained with lineage markers, separated by FACS, and RNA recovered for transcript analysis at 0, 7, 11, and 14 d postinfection. The following cell types were identified for cell sorting: myeloid cells (CD45$^+$CD11b$^+$), lymphoid cells (CD45$^+$CD11b$^-$ (B220$^+$ or TCRβ$^+$)), endothelial cells (CD45$^-$CD31$^+$) with many of these also expressing high levels of Ly6C$^+$; and a final group was comprised of a heterogeneous mixture of stromal cell types, such as fibroblasts, chondrocytes, and smooth muscle cells that were CD45$^-$CD31$^+$. A subset of this group was CD90$^+$CD29$^+$, indicative of joint fibroblasts, including synovial fibroblasts, and some of which were also Ly6C$^+$ (data not shown). Pilot studies confirmed that the earliest time to reproducibly capture the IFN profile in cells sorted from the infected joint was day 7 of infection, which coincides with the earliest time point at which B. burgdorferi 16S rRNA can be reliably detected in this tissue (data not shown). IFN-inducible transcripts peaked at 7 d postinfection, in both the unfractonated and each of the sorted populations of joint cells, shown for Cxcl9, Iggp, Gbp2, and Oasl2 (Fig. 6). IFN-induced transcript levels receded dramatically by day 11 postinfection, similar to uninfected levels. In addition to the expected contribution of myeloid cells and lesser contribution of lymphoid cells, endothelial and fibroblast-enriched fractions displayed robust upregulation of the IFN-inducible transcripts. Although the importance of synovial fibroblasts in the pathogenesis of RA is well appreciated (43), the dominating role of endothelial cells and fibroblasts in the tissue response to B. burgdorferi infection has not been previously demonstrated. The precise and synchronized timing of the IFN response in all cell lineages further indicates carefully orchestrated expression patterns in the joint tissue.

The results of Fig. 6 implicated both endothelial cells and joint fibroblasts in the early response to B. burgdorferi in the joint tissue. An interesting observation from the transcript analysis in Fig. 6 is the finding that both Iggp and Gbp2 were constitutively expressed in endothelial cells at higher concentrations than found in myeloid or fibroblast-enriched cells. This suggested the possibility that endothelial cells of the joint tissue were poised to respond to blood-borne pathogens or inflammatory mediators. Therefore, activation states of endothelial cells and synovial fibroblasts were further dissected with more specific staining reagents, and the FACS separation was repeated for joint tissue from uninfected and day 7 infected C3H mice. Leukocytes were identified as CD45$^+$, endothelial cells were identified as CD31$^+$CD45$^-$, and in this protocol joint fibroblasts were isolated using the markers CD45$^+$ and CD31$^-$ to remove hematopoietic and endothelial cells, followed by enrichment for fibroblasts, including synovial fibroblasts, using CD90$^+$CD29$^+$ (Fig. 7A) (44). The fidelity of the sorting protocol was confirmed by lineage-specific transcript analysis and revealed enrichment of CD45 transcripts only in leukocytes, CD31 enrichment in endothelial cells, CD90 (Thy1) enrichment in fibroblasts and leukocytes, and enrichment of fibronectin in joint fibroblasts and other cell types, which were not defined in our analysis, but include epithelial cells, chondrocytes, and smooth muscle cells (Fig. 7B). Transcripts from Mmp3 were also only identified in the joint fibroblast and other fractions (data not shown), further evidence that this represents a functionally discrete subset in the joint tissue. Similar confirmation of sorting fidelity was obtained for the lineage-sorted fractions used in Fig. 6 (data not shown). The activation status of endothelial cells and fibroblasts in the joint at the critical day 7 time point was studied following FACS recovery of cells stained for VCAM1, ICAM1, and PECAM1. Endothelial cells demonstrated increased staining intensity and transcriptional upregulation for all three activation markers at day 7 postinfection.

**FIGURE 6.** Ex vivo identification of endothelial cells and fibroblasts as major contributors to the IFN response of infected C3H joints. FACS analysis of cells released from joint tissue of C3H mice infected for 0 (media), 7, 11, and 14 d. Single-cell suspensions of joint tissue were prepared for lineage staining, and FACS was used to collect and quantify lineages. RT-PCR analysis of sorted lineages at each time point ($n = 3$). Myeloid cells (CD45$^+$CD11b$^+$), lymphoid cells (CD45$^+$CD11b$^-$, TCRβ$^+$, or B220$^+$), endothelial cells (CD45$^-$CD31$^+$), and fibroblast-enriched cells (CD45$^-$CD31$^+$) were sorted simultaneously. Expression of IFN-inducible transcripts, Gbp2, Iggp, Cxcl9, and Oasl2, was normalized to β-actin. Statistical significance between experimental and control groups (*$p < 0.05$) is indicated.
whereas joint fibroblasts showed increased expression of Vcam1 and Icam1 (and do not express Pecam1) (Fig. 8A, 8B). These data indicate that cellular activation precedes proliferation of endothelial cells and fibroblasts shown earlier (Fig. 5B), and demonstrate strong correlation between transcript induction and protein expression at day 7 postinfection. Interestingly, these VCAM1\^ICAM1\^ fibroblasts were also Ly6C\^ (data not shown), and may constitute fibroblast-like synoviocytes implicated in RA (43, 44).

Transcriptional analyses of the highly enriched endothelial and fibroblast fractions from the joint further supported their contribution to the IFN profile at day 7 (Table II). Endothelial cells and fibroblasts were found to be major contributors of the classic IFN-inducible transcripts Gbp2, Iigp, and Oasl 2, as well as the IFN transcriptional activator Stat1. The contribution of endothelial cells and fibroblasts to the B. burgdorferi-induced IFN response is striking, and previously unrecognized, although human endothelial cell cultures were previously shown to respond to B. burg-
through a classic NF-κB–dependent signaling cascade (45). Further analyses of the transcriptional response to *B. burgdorferi* revealed both joint fibroblasts and endothelial cells to be the dominant sources of chemokines (Table III), with joint fibroblasts supplying the PMN and monocyte-recruiting Cxcl1, Cxcl2, Ccl2, and Ccl8, and endothelial cells serving as the primary source of the CXCR3-interacting chemokines Cxcl9 and Cxcl10, important in recruiting NK and T cells. This coincides with an increase in the expression of the classic activation markers VCAM1, ICAM1, and PECAM1 by fibroblasts and endothelial cells (Fig. 8), which would further contribute to the recruitment of inflammatory cells. Also of note is the production of Cxcl1 and Cxcl10 by endothelial cells, important stimulants for neutrophils and activated neutrophils, respectively. The unique contribution of leukocytes to Cxcl13 recruitment of B lymphocytes may be important in the resolution of infection and disease. It is particularly interesting in light of the strong association of CXCL13 with tissue-specific infection by *B. burgdorferi* in humans (46, 47).

Integration of our ex vivo analysis of the early responses of the infected joint tissue with characteristics of the arthritic lesions at 28 d postinfection has allowed development of a dynamic model for *B. burgdorferi*-induced arthritis development in C3H mice (Fig. 9). Two phases of arthritis development are shown in this model, with the first phase incorporating the initiation of type I IFN production and the upregulation of other proinflammatory molecules, and the second phase depicting the progression to the arthritic lesion. The involvement of myeloid cells, endothelial cells, and fibroblasts is depicted, with upregulation of chemokines by fibroblasts and synoviocytes providing the key stimulus for arthritis development.

**Discussion**

Previously, we noted a transient and early induction of type I IFN signature transcriptional response in the joint tissue of *B. burgdorferi*-infected C3H mice and determined this to be a predictor of the severity of Lyme arthritis in this mouse strain (9). Subsequently, we discovered that blocking the early type I IFN signaling cascade by systemic administration of mAb muted the arthritic response in C3H mice at 4 wk postinfection, thus formally coupling the induction of type I IFN to Lyme arthritis (13). Together these findings defined a model by which joint-localized bacteria triggered a provocative burst of type I IFN that in turn established the IFN-inducible arthritis-dependent pathway. Both CD45+ and CD45− cells responded to exogenous IFN-β by amplifying the production of IFN-inducible transcripts, indicating their potential contribution to the arthritis-associated response. Both CD45+ and CD45− cells of the naive joint upregulated Tnfa in response to *B. burgdorferi* in vitro, simulating the potential contribution of IFN-independent signaling pathways during infection.

The ability of human and murine macrophages, monocytes, and dendritic cells to initiate a type I IFN response when stimulated with *B. burgdorferi* in vitro has been clearly documented; how-

<table>
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<th>Cell Type</th>
<th>Treatment</th>
<th>Gbp2</th>
<th>Igp</th>
<th>Oasl2</th>
<th>Stat1</th>
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<td>Media</td>
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<td>$116 \pm 30.1$</td>
<td>$3 \pm 1.7$</td>
<td>$13 \pm 5.7$</td>
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<tr>
<td>Other</td>
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<td>$514 \pm 62.9$</td>
<td>$44 \pm 7.1$</td>
<td>$160 \pm 12.7$</td>
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<tr>
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<td>$11 \pm 1.9$</td>
<td>$3 \pm 1.5$</td>
<td>$6 \pm 1.7$</td>
</tr>
<tr>
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<td>$38 \pm 11.1$</td>
<td>$71 \pm 23.8$</td>
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<td>$149 \pm 20.7$</td>
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<td>$53 \pm 7.8$</td>
</tr>
</tbody>
</table>

*aRNA prepared from lineage-sorted joint cells at day 7 of infection.

*bMean ± SE for samples from four mice, normalized to β-actin.

**Bolded numbers indicate greater induction compared with mice treated with media alone, p < 0.05.**
ever, to our knowledge, this is the first study to directly assess the potential of cells of the joint tissue to mount this response. Our results are most consistent with a resident mononuclear cell initiating the IFN profile following phagocytosis of *B. burgdorferi* and processing *Borrelia* ligands capable of activating cellular sensors. Numerous laboratories studying the IFN response to *B. burgdorferi* have identified MyD88-dependent sensors, particularly TLR7/8, and MyD88-independent, IFN regulatory factor-3–dependent sensors capable of inducing type I IFN in cultures of mouse and human mononuclear cells (34-37, 50, 51). *B. burgdorferi* ligands implicated in this response include RNA, lipopolysaccharides, and secreted bacterial components (25, 34, 36, 52). Interestingly, the Ly6C+ inflammatory monocytes, whose recruitment has been implicated in the IFN response in other systems (41, 42, 53), were not increased in joint tissue until day 11 postinfection and, therefore, this expansion was not required for the day 7 IFN signal amplification (Fig. 5). The appearance of Ly6C+ cells may have greater impact on later responses to *B. burgdorferi*, which shift to classic NF-κB–dependent production of cytokines and chemokines on days 11 and 14 postinfection (9).

Ex vivo analysis of cells recovered from joints of infected C3H mice revealed cell type contributions to the IFN transcriptional signature at day 7 postinfection. Whereas the contribution of myeloid cells to the profile was expected, the magnitude of the contribution made by endothelial cells and fibroblasts of the joint tissue was a surprise. Based on our finding that CD45+ cells of joint of naïve mice did not upregulate the IFN profile in response to *B. burgdorferi* alone but did respond well to exogenous IFN-β, we suspect that the robust responses of endothelial cells and joint fibroblasts from infected mice reflect the combined effects of *B. burgdorferi* and the autocrine/paracrine activity of type I IFN, together resulting in exuberant production of amplified type I IFN and downstream products (Figs. 4, 6, 8, Table II). The upregulation of *Stat1* transcripts at day 7 postinfection is consistent with the importance of the IFN receptor-dependent amplification stage of this response, even at this early time point.

Of particular importance to the development of Lyme arthritis was the finding that fibroblasts of the joint were the major producers of chemokines involved in recruitment of the hallmark cell of Lyme arthritis, the PMN (Table III). Consistent with these data is the documented role of fibroblast-like synoviocytes in promoting inflammation in RA (54). Brown and colleagues (55, 56) previously demonstrated the critical role of PMN-recruiting chemokines and their receptors in the development of Lyme arthritis, with monocytic chemokines playing an analogous role in carditis development. Our results point to the activated fibroblast (likely synovial) as the source of PMN and monocye-recruiting chemokines in Lyme arthritis development and resolution. The relative contribution of fibroblasts occupying the joint space versus synoviocytes comprising the membranous synovial sheath could not be determined in this study. These findings indicate that activation of endothelial cells and fibroblasts in the joint sets the stage for subsequent recruitment of arthritis-defining inflammatory cells, as shown in phase 1 of Fig. 9. The importance of additional inflammatory pathways in the sustained recruitment resulting in arthritis development is suggested by the residual Lyme arthritis seen in C3H IFNAR1−/− mice as well as the ability of CD45+ cells from the joint tissue to upregulate transcripts for TNF-α in response to *B. burgdorferi*. The development of severe Lyme arthritis is clearly influenced by multiple pathways activated simultaneously, with the full-blown lesion observed in C3H mice reflecting the combined effects. Fundamental to our model is the requisite involvement of joint-localized bacteria in every stage of lesion development.

Our findings suggest a model by which *B. burgdorferi* exits the vascular endothelium and enters the joint tissue, potentially encountering several types of cells (Fig. 9) (57, 58). Endothelial cells may be the first to encounter *B. burgdorferi* as it exits the blood, and human endothelial cells are known to engage TLR-MyD88–dependent signaling in response to *B. burgdorferi* (45). Fibroblasts are also abundant components of connective tissue of the joint, and the interaction of *B. burgdorferi* with synovial fibroblasts, fibrocytes, and extracellular matrix components of connective tissue has been well established (59, 60). Myeloid cells residing in the joint, possibly including macrophage-like synoviocytes and tissue macrophages, appear to trigger the type I IFN response (Fig. 9). This response most likely requires phagocytosis of *B. burgdorferi* and liberation of bacterial components that result in IFN production (9). This response most likely requires phagocytosis of *B. burgdorferi* and liberation of bacterial components that result in IFN activation and downstream products (Figs. 4, 6, 8, Table II). The upregulation of *Stat1* transcripts at day 7 postinfection is consistent with the importance of the IFN receptor-dependent amplification stage of this response, even at this early time point.

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These observations are striking due to the association of excessive production of type I IFN with other inflammatory-based diseases, including patients with SLE and those receiving therapeutic doses of IFN-αβ for hepatitis and multiple sclerosis (14–16, 61). The recent recognition of an IFN signature response in a
subgroup of RA patients that fail to respond to targeted TNF-α blockade further suggests our findings may be broadly applicable to newly recognized patient groups (18, 19, 62). We propose the IFN-dependent Lyme arthritis in C3H mice to be a model to study the complex interactions that result in tissue-specific and systemic activation of pathological concentrations of type I IFN. We further propose that the similarities with RA, particularly the involvement of synovial fibroblasts in the amplification of the inflammatory response and recruitment of inflammatory cells, are highly relevant to disease development, and that understanding the dynamics of initiating and amplifying populations in pathological responses may have broad implications for inflammatory joint diseases.

**FIGURE 9.** Proposed mechanism of injury in joint tissue of C3H mice infected with *B. burgdorferi*. Phase 1: Initiation of the localized inflammatory response. (A) Spirochetes migrate to joint tissue, triggering activation of endothelial cells and fibroblasts and upregulation of adhesion molecules. (B) Resident myeloid lineage cells, such as macrophages, phagocytose *B. burgdorferi*, triggering production of IFN-β (C). (D) IFN-β signal is amplified in an autocrine/paracrine fashion, involving a large number of cell types, including endothelial cells and fibroblasts. (E) Synoviocytes and endothelial cells produce a variety of chemokines, leading to a chemotactic gradient. Increased adhesion molecule expression potentiates leukocyte migration into tendon sheath and joint capsule. Phase 2: Progression to the joint lesion characteristic of Lyme arthritis (14–28 d). (F) Increased expression of cell adhesion molecules on vascular endothelial cells promotes attachment and extravasation of leukocytes into the extracellular matrix, and myeloid hyperplasia. (G) Fibroblast hyperplasia and increased vascularization develop within the tendon sheath, as well as (H) synoviocyte hyperplasia. IFN-β response is muted by this time point; thus, sustained inflammation most likely reflects effects of classic proinflammatory pathways activated by persisting *B. burgdorferi*. (Figure courtesy of James F. Zachary, University of Illinois-Urbana.)


