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 naïve 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 polymorphonuclear 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 pathogeneses of diseases associated with type I IFN signature, including systemic lupus erythematosus and some rheumatoid arthritides. *The Journal of Immunology*, 2012, 189: 000–000.
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 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 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 × 10^6 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 × 10^6 universal type I IFN (PBL) on day 1 and 10^5 U every other day for 28 d by i.p. injection, or an equivalent volume of PBS (21). Ankle measurements were obtained using a micrometer caliper before and at 4 wk of infection. Rear ankle joints were prepared for assessment of histopathology by removal of skin and fixation of the tissue in 10% neutral buffered formalin, as described (8). Decalcified joints were embedded in paraffin, sectioned at 3µm, and stained with H&E. Each slide was scored from 1 to 5 for various aspects of disease, including severity and extent of the lesion, polymorphonuclear leukocyte (PMN) and mononuclear cell (e.g., monocytes, macrophages) infiltration, tendon sheath thickening (e.g., synovocyte and fibroblast hyperplasia), and reactive/repairative responses (e.g., periosteal hyperplasia and new bone formation and remodeling), with 5 representing the most severe lesion, and 0 representing no lesion. Ankle measurements and arthritic lesions were assessed in coded samples. Infection was confirmed in mice euthanized prior to 14 d postinfection by culturing bladder tissue in BSK II media containing 6% rabbit serum, phosphohypycin, and rifampicin. ELISA quantification of B. burgdorferi-specific IgM and IgG concentrations was used to confirm infection in mice euthanized and at after 14 d postinfection, as described (22).

**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-amoactinomycin D (eBioscience) or DAPI (Invitrogen) was used in all experiments, and dead cells were excluded from analysis, as were doublets. 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-CD54 (YN1/1.7.4), anti-CD31 (590), anti-IFNAR1 (MARI-583), and anti-CD29 (HM51-1); PE/Cy7-conjugated anti-CD11b (M1/70), anti-CD90.2 (30-H12), and anti-CD45.2 (104); alphaloprotein-conjugated anti-F4/80 (BAM8), anti-TCR B (H5-579), anti-CD29 (HM51-1), anti-CD45 (30-F11), and anti-CD104 (429); Alexa Fluor 700-conjugated anti-Ly6G/Ly6C (RB6-8C5) and anti-CD45.2 (104); Pacific Blue-conjugated anti-TACRP (H5-579) and anti-B220 (RA3-6B2); and biotin-conjugated anti-PE (PE001) and PE-conjugated streptavidin.

**Injection of mAbs**

The following Abs were used in vivo for cytokine neutralization: anti–IFN-γ (XMG1.2), anti–TNF-α (XCT.31), and rat IgG1 (HPRN) 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 Roche LC-480 according to our previously described protocols (24). Primer sequences used in this study were as follows: Cxcl2 forward (5′-CCACAATGTTAGCTGGAGAG-3′), reverse (5′-GTTTTTCCGTTGCTCTATCAG-3′); Cxcl8 forward (5′-GGTTTCTTGGTGCGCGTGCTTAAG-3′), reverse (5′-CATTGTGTTAATTCTCTCTGGC-3′); Cxcl11 forward (5′-ATGCTGGTGGATATTCCAATTC-3′), reverse (5′-CCTCCGAACTACAGCGTG-3′); Cxcl22 forward (5′-CCCCCTGGGGCGGAG-3′), reverse (5′-AGGTCAGTTAGTGGCCCTT-3′); Ifn1 forward (5′-GACGTTGTTGACTTCAATGAGGC-3′), reverse (5′-TCTCTTTTCTATTT CGGAG-3′); Icam1 (CD54) forward (5′-GGAGCTCAGTTGTTGTCCTA-3′), reverse (5′-CTTCCGAGGACCAAGAAACC-3′); Pecam1 (CD31) forward (5′-TCTCTTCCATCTACAACGATCC-3′), reverse (5′- TTTGTTGATCTGCTCCCGCCTTT-3′); Ppecam1 (CD45) forward (5′-GGCTCAGTCGTCTCTTCTGC-3′), reverse (5′-GGTCTGCTGGCTCTTCT GCC-3′); Thy1 (CD90) forward (5′-GGATGAGGGGCGGACACCTTGG-3′), reverse (5′- TTGTGGACATGGTCTCCGCTCT-3′); Nidogen forward (5′-GGTC GGTGGGATTCACCTC-3′), reverse (5′-CTTCATAGCTTCACATAGGG-3′); Gbp2 (CD45) forward (5′-GGACCCATGTCATTGGCTC-3′), reverse (5′-GGTCACTACA TGGCTC-3′); Cxcl10b (IFNAR1) forward (5′-GAGTGGAGGGCGGAGACCC-3′), reverse (5′- TGGTGGACATGGTCTCCGCTCT-3′); Ppecam1 (CD45) forward (5′-GGAGCTCAGTTGTTGTCCTA-3′), reverse (5′-CTTCCGAGGACCA AGAACC-3′), Primer sequences for β-actin, Igg, Igk, Mplp3, Stat1, Cxcl13 (9), Cxcl9, Cxcl10, Oasl2 (13), Ifng, Gbp2 (25), Tofa, and Ifox (26) were 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 Nidogen 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−/− mice 5 wk 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 one 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 × 10^4/ml in media containing the serum replacement Nutridoma (Roche) and stimulated with live B. burgdorferi cN40 (7.4 × 10^4/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). Labeled cells were loaded onto MS columns (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 fractionated populations were incubated in 2% FBS containing RPMI 1640 or Bioactive type I IFN was detected in culture supernatants from BMDM (eBioscience) by bioassay with B16-Blue 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.

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 rIFN-γ (eBioscience) by bioassay with B16-Blue IFN-αg 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 t test (Figs. 1, 8, Table I). Categorical data for histopathology were 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/reactive 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).

Effect of type I IFN receptor ablation on B. burgdorferi-induced IFN profile in joint tissue and in macrophage cultures
The contribution of type I IFN signaling to the previously reported robust upregulation of IFN-responsive transcripts was assessed in C3H IFNAR1−/− mice at 1 wk of B. burgdorferi infection. Interestingly, several of the IFN-inducible transcripts previously found to be reduced but not eliminated by receptor-blocking mAb also displayed residual induction in the joint tissue of B. burg-

Table I. Effect of IFN-α administration on arthritis development in B. burgdorferi-infected B6 mice

<table>
<thead>
<tr>
<th>Infection Status</th>
<th>IFN Treatment</th>
<th>Change in Ankle Measurement</th>
<th>Overall Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock infected</td>
<td>PBS</td>
<td>0.01 ± 0.01a</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>B. burgdorferi</td>
<td></td>
<td>0.04 ± 0.04</td>
<td>1.0 ± 0.71</td>
</tr>
<tr>
<td>Mock infected</td>
<td>IFN-α</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>B. burgdorferi</td>
<td></td>
<td>0.25± ± 0.15</td>
<td>1.6 ± 0.55</td>
</tr>
</tbody>
</table>

aValues represent mean ± SD.

*Statistical significance between PBS- and IFN-α-treated group, p < 0.05.
FIGURE 1. IFNAR1 gene ablation results in reduced Lyme arthritis severity in C3H mice. C3H or C3H-IFNAR1−/− mice were infected with B. burgdorferi by intradermal injection, and arthritis was assessed at 4 wk, as described in Materials and Methods. Arthritis traits included the following: change in ankle joint measurement, PMN infiltration, and overall lesion severity. B. burgdorferi number in joint tissue was determined by quantitative PCR and normalized to the single copy mouse gene Nidogen. Statistical significance was determined by Student t test for ankle swelling and bacterial number in joint tissue, whereas the Mann–Whitney U test was used for PMN infiltration and overall lesion. All categories were negative for uninfected control C3H and C3H-IFNAR1−/− mice, injected with BSK media, and are not shown in the figure. n ≥ 9 mice for each group, pooled from two independent experiments. *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 the reduction 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 Tnfα 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 BMDM 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 (n = 5). The following transcripts were analyzed and normalized to β-actin: Cxcl10, Oasl2, Igtp, and Gbp2. (B) Effect of exogenous IFN-γ on transcriptional induction of IFN profile in BMDM from C3H and C3H IFNAR1−/− mice incubated with *B. burgdorferi*, IFN-γ, *B. burgdorferi* plus IFN-γ, or media alone for 6 h. RT-PCR analysis of IFN profile transcripts (Cxcl10, Oasl2, Igtp, and Gbp2), normalized to β-actin. (C) *B. burgdorferi*-induced secretion of bioactive type I IFN protein in culture supernatants of C3H and C3H IFNAR1−/− BMDM, collected at 24 h. Supernatants were applied to type I IFN reporter cell line for IFN bioassay, as described in Materials and Methods. Transcription analysis of Ifnb at 6 h from same experiment is shown for comparison, and Tnfa transcripts are included as viability and responsiveness control. Data are representative of two independent experiments. Statistical significance among groups (transcript analysis) or between experimental and control groups (IFN bioassay) is shown (*p < 0.05).
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-kB–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 upregulating *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 ex 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

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**FIGURE 3.** Radiation chimeras between C3H and IFNAR1⁻/⁻ mice reveal contribution of both resident cells and cells of hematopoietic origin to proarthritic IFN response. Reciprocal radiation chimeras between C3H and C3H IFNAR1⁻/⁻ mice were generated, as described in Materials and Methods. Mice were infected with *B. burgdorferi* 3 wk following reconstitution, and arthritis severity was assessed at 4 wk postinfection, shown for change in ankle measurement and overall lesion score. Direction of transplantation from donor to recipient is indicated on the figure. Results are pooled from two separate experiments, with ≥10 mice in each infected mouse group. *B. burgdorferi* numbers at 4 wk postinfection were similar in all mice. Uninfected chimeras did not display ankle abnormalities or *B. burgdorferi* DNA in tissues. Statistical significance among groups (*p < 0.05) is indicated.

**FIGURE 4.** Potential contribution of hematopoietic and nonhematopoietic cells to the initial IFN response to *B. burgdorferi* in joints of C3H mice. Enrichment of leukocytes in the CD45⁺ fraction was confirmed by flow cytometry (A) with significant change from unfractionated indicated (*p < 0.05). RT-PCR of transcripts for *Tnfa* (B), *Cxcl10* (C), and *Tyki* (D) identified populations responsive to *B. burgdorferi*, IFN-β, and combined treatment, and were normalized to β-actin. Only CD45⁺ cells displayed significant upregulation of the IFN-inducible transcripts *Cxcl10* and *Tyki* in response to *B. burgdorferi* alone, as indicated. Results are representative of three separate experiments, with n = 3. Statistical significance between experimental and control groups (*p < 0.05) is indicated.
determined by flow cytometry assessment of populations released from the joint tissue over time: PMN leukocytes were defined as GR1highLy6CdimCD11bhighCD45+, macrophages were GR1−Ly6C−F4/80−CD11b−CD45−, and inflammatory monocytes were GR1dimLy6ChighCD11b−CD45+ and inflammatory monocytes were GR1dimLy6ChighCD11b−CD45−. 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 IFNAR1−/− mice, indicating the recruitment or expansion of these cells was not dependent on type I IFN signaling. Importantly, the Ly6C+ population showing the greatest increase following infection in both C3H and C3H IFNAR1−/− 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+ 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 IFNAR1−/− 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−CD31+) and fibroblast (CD45−CD31−CD90+CD29+) numbers were increased by days 11 and 14 postinfection in joints from both C3H and C3H IFNAR1−/− 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+ICAM1high) 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 IFNAR1−/− 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+Ly6C+CD11b+CD45+), inflammatory monocytes (Ly6CdimGR1−CD11b−CD45−), and macrophages (GR1−Ly6C−F4/80−CD11b−CD45−) 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−CD31−CD90+CD29+) or endothelial cells (CD45−CD31+) by flow cytometry at days 0 (media), 7, 11, and 14 of infection. Activated cells were identified as VCAM1+ICAM1high. Statistically significant differences were found at 11 and 14 d of infection relative to uninfected mice, for both the C3H and IFNAR1−/− 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.
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 were CD45.2+CD11b+; lymphoid cells were CD45.2−CD11b− (B220+ or TCRβ+); endothelial cells were 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, Igp, 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 Igp 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.
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 Oasl2, 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 synovioocytes 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 arthritis 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 an inflammatory cascade that resulted in severe arthritis 2–3 wk later (Fig. 9). The identification of the cellular interactions required to trigger the response in the joint tissue was important in understanding the initiation of this response in C3H mice. A variety of different cell types have been identified as initiators of type I IFN in other infectious and pathological conditions, suggesting that in vivo analysis would be required to characterize the cells responsible for initiating and executing this response in the joint.

To achieve a more rigorous assessment of type I IFN-dependent signaling on the development of Lyme arthritis, the IFNAR1 gene disruption was crossed onto the C3H mouse. Experiments with this mouse strain confirmed the importance of type I IFN signaling in the development of arthritis (Fig. 1), but also confirmed the importance of additional, IFN-independent pathways in the maximal arthritis of infected C3H mice, in line with the documented multigenic nature of this arthritis (24, 30). The development of the C3H IFNAR1−/− mouse permitted the generation of reciprocal radiation chimeras with C3H mice. These experiments revealed contributions of both hematopoietic and mesenchyme-derived cells to the IFN-dependent portion of arthritis development (Fig. 3), consistent with the broad expression of the type I IFN receptor and its importance in promoting the antiviral state in most lineages (16). Interestingly, type I IFN signaling was not required for effective control of bacteria number in joint or other tissues, a contrast from findings with IFN-γ (Figs. 1, 3) (10, 11, 48). This observation raises the possibility that the type I signaling pathway could provide a novel therapeutic target for Lyme arthritis without disrupting the efficacy of ongoing antibacterial treatments.

It is generally accepted that *B. burgdorferi* can enter tissues following hematogenous spread, and that it is the response of the host to bacteria in the tissue that initiates an inflammatory response (5, 49). Therefore, a more precise ex vivo analysis of cells from the joint tissue was employed to assess the cellular dynamics of the response to *B. burgdorferi* invasion. Consistent with the radiation chimera experiment, both hematopoietic cells and fibroblasts and endothelial cells of the joint contributed to the robust IFN profile in infected joint tissue (Figs. 4, 6, Tables II, III). Evaluation of cells recovered from the naive joint revealed a much more limited ability to generate an IFN response to *B. burgdorferi*: only CD45+ cells had this capability. However, 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>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment</th>
<th>Gbp2</th>
<th>Isgp</th>
<th>Oasl2</th>
<th>Stat1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte</td>
<td>Media</td>
<td>2 ± 0.6&lt;br&gt;Infected</td>
<td>ND</td>
<td>3 ± 0.8</td>
<td>10 ± 2.9</td>
</tr>
<tr>
<td>Endothelial</td>
<td>Media</td>
<td>29 ± 3.5&lt;br&gt;Infected</td>
<td>13 ± 3.7</td>
<td>19 ± 5.6</td>
<td>21 ± 4.2</td>
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<tr>
<td>Fibroblast</td>
<td>Media</td>
<td>36 ± 9.0&lt;br&gt;Infected</td>
<td>116 ± 30.1</td>
<td>3 ± 1.7</td>
<td>13 ± 5.7</td>
</tr>
<tr>
<td>Unsorted</td>
<td>Media</td>
<td>447 ± 22.3&lt;br&gt;Infected</td>
<td>514 ± 62.9</td>
<td>44 ± 7.1</td>
<td>160 ± 12.7</td>
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</table>

*Bolded numbers indicate greater induction compared with mice treated with media alone, p < 0.05.*
Table III. Endothelial cells and fibroblasts upregulate various chemokine transcripts in joints of B. burgdorferi-infected C3H mice

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment</th>
<th>Ccl9</th>
<th>Ccl10</th>
<th>Ccl11</th>
<th>Ccl12</th>
<th>Ccl13</th>
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<tr>
<td>Leukocyte</td>
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<td>0.7 ± 0.4</td>
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<td>3 ± 0.8</td>
<td>54 ± 13.2</td>
<td>0.3 ± 0.3</td>
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<tr>
<td>Infected</td>
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<td>5 ± 2.4</td>
<td>13 ± 4.9</td>
<td>9 ± 4.0</td>
<td>43 ± 10.8</td>
<td>6 ± 0.9</td>
</tr>
<tr>
<td>Endothelial</td>
<td>Media</td>
<td>7 ± 2.9</td>
<td>9 ± 8.6</td>
<td>6 ± 3.1</td>
<td>4 ± 2.4</td>
<td>ND</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td>156 ± 11.2</td>
<td>124 ± 21.4</td>
<td>25 ± 3.6</td>
<td>4 ± 1.2</td>
<td>1.3 ± 1.3</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>Media</td>
<td>0.5 ± 0.5</td>
<td>3 ± 1.6</td>
<td>61 ± 3.2</td>
<td>9 ± 3.0</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td>87 ± 33.0</td>
<td>119 ± 15.9</td>
<td>233 ± 30.6</td>
<td>49 ± 16.3</td>
<td>7 ± 3.5</td>
</tr>
<tr>
<td>Other</td>
<td>Media</td>
<td>ND</td>
<td>2 ± 1.9</td>
<td>13 ± 3.3</td>
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</tr>
<tr>
<td>Infected</td>
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<td>16 ± 8.5</td>
<td>44 ± 7.7</td>
<td>36 ± 5.9</td>
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<td>ND</td>
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<td>Media</td>
<td>4 ± 0.5</td>
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<td>Infected</td>
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<td>31 ± 1.9</td>
<td>24 ± 2.2</td>
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*RNA prepared from lineage-sorted joint cells at day 7 of infection.

*Mean ± 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, lipopolysaccharide, 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-kB–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 types contributing 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 naive 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 monocyte chemokines playing an analogous role in carditis development. Our results point to the activated fibroblast (likely synovial) as the source of PMN and monocyte-recruiting chemokines in Lyme arthritis development and resolution. The relative contribution of fibroblasts occupying the joint space versus synoviocytes comprising the membrane of 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 regulatory factor-3 activation and type I IFN induction (25, 35, 36, 38, 50). Once type I IFN production is initiated, numerous cell types of the joint, particularly endothelial cells and synovial fibroblasts, engage the type I IFN receptor and join the IFN signature response, leading to a positive feedback that amplifies the signaling cascade and sets the stage for the development of severe Lyme arthritis, phase 2 (Fig. 9).

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-α/β 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.)
Disclosures

The authors have no financial conflicts of interest.

References


