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A critical role for Type I IFN in arthritis development following *Borrelia burgdorferi* infection of mice

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Abstract

Gene expression analysis previously revealed a robust IFN-responsive gene induction profile that was selectively upregulated in *Borrelia burgdorferi*-infected C3H mice at 1 week post-infection. This profile was correlated with arthritis development, as it was absent from infected, mildly arthritic C57BL/6 mice. In this report, we now demonstrate that profile induction in infected C3H *scid* mice occurs independent of B or T lymphocyte infiltration in the joint tissue. Additionally, Type I IFN receptor-blocking antibodies, but not α -IFN γ antibodies, dramatically reduced arthritis, revealing a critical but previously unappreciated role for Type I IFN in Lyme arthritis development. Certain examined IFN-inducible transcripts were also significantly diminished within joint tissue of mice treated with α -IFNAR1, whereas expression of other IFN-responsive genes was more markedly altered by α -IFN γ treatment. These data indicate that induction of the entire IFN profile is not necessary for arthritis development. These findings further tie early Type I IFN induction to Lyme arthritis development, a connection not previously made. Bone marrow derived macrophages readily induced IFN-responsive genes following *B. burgdorferi* stimulation, and this expression required functional Type I IFN receptor. Strikingly, induction of these genes was independent of TLRs 2,4, and 9, and of the adapter molecule MyD88. These data demonstrate that the extracellular pathogen *B. burgdorferi* utilizes a previously unidentified receptor and a pathway traditionally associated with viruses and intracellular bacteria to initiate transcription of Type I IFN and IFN-responsive genes and to initiate arthritis development.

Keywords

Rodent; monocyte/macrophage; natural killer cells; bacterial; inflammation

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Introduction

Lyme disease is caused by the tick-borne spirochete *Borrelia burgdorferi* (1). Patients afflicted with *B. burgdorferi* can display a wide range of clinical symptoms including erythema migrans (EM), a skin rash at the site of the tick bite, various neurological sequelae, carditis, and arthritis. Although appropriate antibiotic treatment cures Lyme disease, approximately 60% of all individuals who do not receive therapeutic intervention develop arthritis (2–4). This subacute arthritis can be effectively modeled using inbred mice, as susceptible strains such as C3H develop severe arthritis in the rear ankle joints by 4 weeks post-*B. burgdorferi* infection, while other strains such as C57BL/6 develop milder arthritis (5–7). Differences in the arthritis severity of these two strains are not due to discrepancies in host defense, as both harbor similar numbers of spirochetes within their ankle joints (7). Spirochetes are detectable within the arthritic joints of patients and although components of the adaptive immune response, especially antibodies, are important for disease resolution, *scid* and *rag*^{−/−} mice develop severe arthritis, indicating that B and T cells are dispensable for disease development (8,9). While the TLR2-dependent inflammatory response to the numerous lipoproteins that decorate the outer surface of these bacteria has been implicated in arthritis development (2,10), both TLR2^{−/−} and MyD88^{−/−} mice still develop arthritis after *B. burgdorferi* infection (11–14). A clear consensus on the role of NFκB-dependent cytokines in arthritis development has not been reached, as numerous studies aimed at addressing this question have yielded conflicting results (10).

In an attempt to uncover novel pathways regulating arthritis development in C3H, C57BL/6, and C57BL/6 IL-10^{−/−} mice, a previous study conducted in our laboratory utilizing Affymetrix microarray analysis found that the majority of genes induced within the rear ankle joints of C3H mice at 1 week post-*B. burgdorferi* infection were annotated as IFN-responsive. An IFN signature was also observed within the ankle joints of the IL-10^{−/−} mice, albeit with delayed induction kinetics, as this profile was not upregulated until 2 weeks post-infection (15). Additionally, IFN-responsive genes were also induced within the joints of TLR2^{−/−} mice on both the C3H and C57BL/6 backgrounds by 1 week following *B. burgdorferi* infection (16). While IFNγ has been shown to be dispensable for arthritis development in C3H mice (17), the role of Type I IFN in the generation of Lyme arthritis has not been assessed. There is precedent for consideration of the impact of Type I IFN on Lyme arthritis development, as arthritis is a documented side effect of therapeutic administration of Type I IFN to Hepatitis C and multiple sclerosis patients (18,19). Several studies have also established a connection between the production of Type I IFN and the development of systemic lupus erythematosus (20–23). In addition, Gattorno and colleagues recently described the accumulation of Type I IFN-producing cells, with a plasmacytoid dendritic cell-like morphology, in the synovial fluid and inflamed tissue of juvenile idiopathic arthritis (JIA) patients (24). These data suggest that the production of Type I IFN may be associated with the development of rheumatoid arthritis in children. Finally, there is evidence that Type I IFN can be produced by humans in response to *B. burgdorferi* infection, as IFNα was detected in peripheral blood and blister fluid obtained from Lyme disease patients with multiple EM lesions (25).

This report explores the impact of Type I IFN depletion on the development of arthritis in C3H mice, as a test of the hypothesis that production of Type I IFN by 1 week post-infection sets the stage for the resultant severe arthritis phenotype exhibited by these mice at later time points.

Materials and Methods

Bacterial culture and mouse infection

C3H/HeN mice were obtained from Charles River Breeding laboratories or NCI, depending on availability. C57BL/6 mice were purchased from NCI, and C3H/HeJ and C3H SCID mice were obtained from Jackson Laboratories. C3H TLR2^{-/-} mice were originally generated by Deltagen Inc., obtained from Tularik, Inc. (now Amgen Inc., South San Francisco, CA) (26). C57BL/6 MyD88^{-/-} mice were kindly provided by Dr. Shizuo Akira (27), and C57BL/6 TLR9^{-/-} were obtained from the Mutant Mouse Resource at the University of California at Davis. C57BL/6 IFNAR1^{-/-} mice were a kind gift from Dr. Murali-Krisna Kaja (University of Washington, Seattle, WA). All mice were housed at the University of Utah Animal Research Center and utilized with strict adherence to all institutional policies and guidelines established for the care and use of laboratory animals in biomedical research.

A clonal isolate of *Borrelia burgdorferi* strain N40 (cN40), kindly provided by Steve Barthold (University of California at Davis) (6), was propagated in BSKII media (28). For infection experiments, 2000 cN40 spirochetes were intradermally injected into the shaved back of 6 wk old, female mice in a volume of 20 μ l. Uninfected, control mice received 20 μ l of BSKII. Mice were sacrificed at 1, 2, or 4 wks post-infection and ankle measurements taken with a metric caliper were compared with pre-infection measurements and recorded as the change in ankle measurement (29). For mock-infected control mice, as well as animals euthanized at 1 and 2 wks post-infection, all skin was carefully removed, and both rear ankle joints were excised and separately snap frozen in a dry ice-ethanol bath for subsequent RNA extraction and RT-PCR analysis of IFN-inducible genes.

At 4 wks post-infection, the most swollen ankle joint, as assessed by metric caliper measurement, was excised and placed in 10% neutral-buffered formalin (Sigma). Samples were then decalcified, paraffin-embedded, sectioned, and stained with hematoxylin and eosin by AML Laboratories (Rosedale, MD). Arthritis severity was assessed in a blinded fashion, and overall scores for joints obtained from both mock-infected and *B. burgdorferi*-infected mice were assigned based on a scale of 0 (no arthritis) to 5 (most severe arthritis), according to the following parameters: overall lesion appearance, degree of neutrophil infiltration, amount of monocytic infiltration, tendon sheath thickness, and the presence of reactive and reparative responses. The least swollen joint was removed at 4 weeks post-infection for quantitative PCR assessment (qPCR) of *B. burgdorferi* numbers within the joint.

Antibodies and depletion protocols

The following antibodies were purchased from eBioscience and utilized for flow cytometry analyses: FITC-conjugated α -DX5 (CD49b) pan-NK cell antibody, PE-conjugated α -CD3e, PE-conjugated α -Armenian hamster IgG, FITC-conjugated α -rat IgM, FITC-conjugated α -CD11c, PE-conjugated α -B220, and FITC- α -Hamster IgG. PE- α -Rat IgG2a was purchased from BD Pharmingen.

MAR1-5A3, a mouse anti-mouse IgG1 monoclonal antibody directed against IFNAR1 was used to inhibit Type I IFN responses in C3H/HeN mice (30). The mouse IgG1 monoclonal antibody MOPC-21 (BioExpress Cell Culture Services, West Lebanon, N.H.) or the mouse anti-human IFNGR1 antibody GIR-208 (31) was utilized as an isotype control. Groups of 5 age-matched, female C3H/HeN mice received an intraperitoneal injection of PBS, or 2.5 mg of either MAR1-5A3, or an appropriate isotype control, one day prior to *B. burgdorferi* infection. IFN γ was neutralized using the hamster H22 monoclonal antibody (32). The hamster α -GST monoclonal antibody PIP was used as an isotype control. C3H/HeN mice

were injected intraperitoneally with 0.5 mg of either H22 or PIP, or an equal volume of PBS, two days prior to *B. burgdorferi* infection. 0.25 mg of H22 or PIP, or PBS was administered on the day of infection (day 0), and booster injections were given every seven days thereafter for the duration of the study. To block both Type I IFN and IFN γ responses, intraperitoneal injections of either 2.5 mg MAR1-5A3 + 0.5 mg H22, 2.5mg MOPC-21 + 0.5 mg Hamster IgG, or an equal volume of PBS, were administered one day prior to *B. burgdorferi* infection. The rabbit polyclonal antibody α -asialo GM1 (Wako Chemicals, Richmond, VA) was utilized to deplete NK cells from C3H mice (33). Groups of 5 female, age-matched mice received 50 μ l (1.8 mg) intraperitoneal injections of α -asialo GM1, rabbit IgG (Sigma), or an equal volume of PBS on the day of infection (day 0), and on day 4. α -asialo GM1 administration resulted in a 75% depletion of NK and NK T cells from the spleen, as assessed by flow cytometry utilizing the FITC-conjugated α -DX5 (CD49b) pan-NK cell antibody. Plasmacytoid dendritic cells (pDCs) were blocked with the α -PDCA-1 monoclonal antibody (Miltenyi Biotec). Groups of 10 female, age-matched mice were injected intraperitoneally with 500 μ g of α -PDCA-1, rat IgG, or an equal volume of PBS one day prior to *B. burgdorferi* infection. Animals were then boosted with 250 μ g of α -PDCA-1 or appropriate controls on the day of infection.

RNA extraction and RT-PCR analysis

Rear ankle joints excised from *B. burgdorferi*- and 16 mock-infected mice were homogenized in chilled guanidine thiocyanate (Roche), and RNA was extracted via cesium chloride ultracentrifugation (34). RNA was extracted from cultured cells with Trizol reagent (Invitrogen), according to the manufacturer's instructions. RNA samples were subsequently reverse-transcribed into cDNA, as described elsewhere (15) and LightCycler-based PCR amplification was conducted utilizing the LightCycler FastStart DNA Master^{plus} SYBR green I Kit for the LightCycler 2.0 instrument (Roche), as previously described (35), or the LightCycler 480 (LC480) SYBR green I master mix for the LC480 machine (Roche). The number of transcript copies present in the starting template sample were calculated, and normalized to 1000 copies of mouse β -actin, as mentioned elsewhere (35). The following primer pairs span introns and were used to amplify cytokines and IFN-responsive transcripts:

*Oasl2*F: TGGGAAGAAGAAAGGGATGGG,

*Oasl2*R: GGGTTTGTCCAGAGTGTCCAATC;

*Cxcl9*F: TTGGGCATCATCTTCCTGGAGCAG,

*Cxcl9*R: GAGGTCTTTGAGGGATTTGTAGTGG;

(LC only) *Il-6d*F: ACAACCACGGCCTTCCCTACTT (36),

*Il-6b*R: TCTCTGAAGGACTCTGGCTTTGTC; (LC480 only)

*Il-6*F: ACCGCTATGAAGTTCCTCTCTGC,

*Il-6d*R: CCAGAAGACCAGAGGAAATTTTC;

*Il-12p40*F: AGATGACATCACCTGGACCT,

*Il-12p40*R: GCCATGAGCACGTGAACCGT. The primer sequences for β -actin, *Igtp*, *Igpl* and 16S rRNA have been reported previously (15,37).

DNA extraction and quantitative PCR (qPCR) analysis

DNA was extracted from mouse tissues, as described elsewhere (38), employing sequential digestions with a 0.1% collagenase A solution and a 0.2 mg/ml proteinase K solution. Following a series of phenol/chloroform extraction and ethanol precipitation steps, the

purified DNA was diluted to 5 µg/ml for use in qPCR assays. Continuous fluorescence monitoring PCR with SYBR green I as the detection dye (Molecular Probes) was conducted with the *ntm17* primer pair and normalized to 1000 copies of mouse *nidogen*, as previously described (38).

Collagenase release of ankle cells

To release cells from the ankle tissue, both rear ankles were excised from uninfected, 10, or 14 days post-*B. burgdorferi*-infected C3H or C57BL/6 mice following skin removal and incubated for 2 hours at 37°C in RPMI-1640 (Invitrogen) containing 10% fetal calf serum (FCS) and 1 mg/ml Collagenase A (Roche), as described previously (16). Two color flow cytometry analysis was conducted to assess whether the numbers of conventional (cDCs) (CD11c+B220⁻) or plasmacytoid (pDCs) (CD11c+ B220⁺) dendritic cells increase in the ankle joints following *B. burgdorferi* infection. The increase in cDC and pDC cell numbers was calculated relative to values obtained for uninfected mice.

Cell culture

Bone-marrow derived macrophages were isolated from the femurs and tibias of mice, as previously described (39), by culture in RPMI-1640 media (Invitrogen) supplemented with 30% L929 conditioned medium and 20% horse serum (Hyclone), demonstrated to be free of *Mycoplasma* with the MycoProbe *Mycoplasma* detection kit (R&D Systems). Harvested macrophages were replated in 6 well dishes at a density of 6×10^5 /ml in media lacking serum and containing 1% Nutridoma. After an overnight incubation, the media was removed and replaced with media alone or media containing 7.4×10^6 /ml live *B. burgdorferi* cN40 (washed twice in PBS to remove BSKII components). Macrophages were stimulated at 37°C, 5% CO₂ for 24 hours, after which the cells were harvested for RNA extraction.

To select for conventional dendritic cells utilizing a low-density protocol, bone marrow cells were cultured in RPMI-1640 media (Invitrogen) containing GM-CSF (kindly provided by Ray Daynes, University of Utah), as previously described (40). Harvested cells were magnetically labeled with α-CD11c microbeads, applied to MACS columns (Miltenyi Biotec), and the CD11c⁺ cells retained by the column were eluted. The purity of these cells was confirmed via flow cytometry staining using α-CD11c and α-B220 antibodies. On average, 90% of the cells recovered utilizing this protocol were CD11c⁺ B220⁻. 1.5 ml of mDCs (1.5×10^6 cells) and 1.5 ml of media only, media plus 1000 U/ml rIFNβ (PBL Interferon Source), 7.4×10^6 /ml live *B. burgdorferi* (Bb), Bb + IFNβ, 10 µg/ml α-OspA (determined to be negative for *Mycoplasma*) (41), or Bb + α-OspA were added to 17 × 100 mm snap-cap tubes (Falcon) and incubated for 24 hours at 37°C, 5% CO₂. Cells were then collected by centrifugation, and RNA was extracted.

pDCs were isolated from the spleens of mice that had received daily intraperitoneal injections of rFlt3L (BioExpress Cell Culture Services, West Lebanon, N.H.) for 9 days (42). Spleens were removed and dissected into RPMI-1640 media containing 2 mg/ml Collagenase A and 20 µg/ml DNase I. pDCs were magnetically labeled with α-PDCA1 microbeads (Miltenyi Biotec) and recovered via separation over a MACS column, followed by elution of PDCA1⁺ cells. Purity was assessed via flow cytometry analysis utilizing α-CD11c and α-B220 antibodies. On average, 95% of the cells recovered utilizing this protocol were CD11c⁺ B220⁺. pDCs were seeded into 24 well plates at 7×10^5 /ml in 1 ml of either complete media, or media containing the following stimuli: 100 U/ml rIFNα (PBL Interferon Source), 7.4×10^6 /ml live *B. burgdorferi* (Bb), Bb + IFNα, 10 µg/ml α-OspA (41), or Bb + α-OspA. Following a 24 hour incubation at 37°C, 5% CO₂, cells were harvested for RNA extraction.

Statistics

The non-parametric Mann-Whitney U-test was utilized for data values obtained from PCR, RT-PCR, and histopathological scoring of arthritis lesions. One-way ANOVA with the Bonferroni post-test was utilized to assess the significance of data obtained from ankle measurements and collagenase-digested ankle joints. All statistical analyses were conducted using GraphPad InStat3 version 3.0b for MacIntosh (GraphPad Software, San Diego, CA). For all tests, $p < 0.05$ was considered significant.

Results

The adaptive immune response is not required for induction of IFN-profile genes in C3H mice

Previous studies demonstrated that a large number of IFN-responsive genes were significantly upregulated by 1 week post-*B. burgdorferi* infection within the rear ankle joints of arthritis-susceptible C3H mice, but not in mildly arthritic C57BL/6 mice, (15). Elevated transcription of IFN-responsive genes could be linked to the severe arthritis phenotype manifested by C3H, but not C57BL/6, mice at later timepoints. Alternatively, triggering of these genes might occur as a result of early infiltrating B and T lymphocytes, which have been reported to secrete IFN γ in response to *B. burgdorferi* (43–46). To distinguish between these two scenarios, C3H *scid* mice, which develop severe arthritis (9), were infected with *B. burgdorferi*, sacrificed at 1 week post-infection, and quantitative RT-PCR of selected IFN-responsive genes was conducted on RNA isolated from both rear ankle joints. Figure 1 depicts transcript levels obtained for four IFN-inducible genes: the IFN γ inducible GTPase, *Igtp* (A); the IFN-inducible GTPase 1, *Iigp1* (B); the oligoadenylate synthase-like 2 gene, *Oasl2* (C); and the T cell recruiting chemokine *Cxcl9* (*Mig*) (D). While the majority of these genes have been previously annotated as IFN γ -inducible, increased transcription of these genes can also be triggered by Type I IFN (47–49). These four genes were selected as representatives of the IFN-responsive profile, as Affymetrix microarray analyses revealed these to be among the most highly upregulated genes at 1 week post-infection. For example, both *Igtp* and *Iigp1* expression levels were elevated greater than 100 fold versus values recorded for uninfected mice (15). Each of the four examined IFN-responsive gene transcripts were elevated within the ankle joints of C3H *scid* mice to similar levels, relative to uninfected mice, as those obtained for wild-type C3H mice (Fig. 1). These data indicate that early recruitment/stimulation of adaptive lymphocytes is not responsible for the transcription of IFN-responsive genes within the ankle joints of C3H mice at 1 week post-infection. Instead, induction of these genes is mediated by cells of the innate immune response.

IFNAR1 blockage results in decreased arthritis severity and diminished IFN-responsive gene expression, but does not alter host defense

A previous report by Brown and colleagues demonstrated that C3H IFN γ ^{−/−} mice develop severe arthritis following *B. burgdorferi* infection, indicating IFN γ is not required for Lyme arthritis development (17). However, much controversy has remained in the field about the impact of IFN γ on arthritis development and resolution (10,50,51). When combined with our observation that IFN-responsive gene induction in C3H *scid* mice mimics that seen in wild-type mice (Fig. 1), it is highly probable that transcription of any IFN-inducible gene associated with arthritis development would be triggered by innate immune cells and could be a consequence of Type I IFN. In line with this hypothesis, the contribution of Type I IFN to Lyme arthritis severity in C3H mice at 4 weeks post-infection was assessed utilizing a mouse monoclonal antibody directed against murine IFNAR1, administered 1 day prior to infection. Overall, mice injected with the α -IFNAR1 monoclonal antibody exhibited reduced ankle swelling, compared with values obtained for mice treated with PBS or IgG1.

Individually, a broad spectrum of arthritis phenotypes was noted for joints obtained from IFNAR1-blocked mice ranging from a drastic reduction in ankle swelling for two mice, to an intermediate phenotype for two mice, to moderately severe ankle swelling for one mouse (Fig. 2A). There was significant concordance between ankle swelling measurements and histopathology, as the two mice exhibiting the least amount of ankle swelling also received greatly reduced lesion scores. A significant reduction in lesion pathology was noted in α -IFNAR1-treated mice when compared with IgG1-treated mice (Fig. 2B). α -IFNAR1-injected mice displayed decreased edema, fewer neutrophils, and less thickening of the cranial tibial tendon sheath (Fig. 3D). These observations were in stark contrast to those noted for PBS- or IgG1 treated mice, both of which exhibited extensive edema, neutrophil infiltration, and thickening of the tendon sheath (Fig. 3B, 3C), features which are classic hallmarks of Lyme arthritis (5,6). Similar results were also obtained in another experiment. To determine whether blockage of Type I IFN impacts immunological clearance of *B. burgdorferi*, qPCR was conducted on DNA extracted from ankle joints of α -IFNAR1-injected mice. There was no significant difference in spirochete numbers within the joints of IFNAR1-inhibited mice versus those detected in PBS- or IgG1-treated mice (Fig. 2C).

To determine whether the decrease in arthritis severity observed as a result of IFNAR1-blockage was linked to a suppression of IFN-inducible genes, the effect of α -IFNAR1 pre-treatment in triggering of IFN-responsive genes at 1 week post-infection was also assessed. Transcript levels for all four examined IFN-responsive genes were significantly suppressed within the ankle joints of C3H mice that received the α -IFNAR1 blocking antibody, when compared with message levels obtained for infected mice treated with either PBS or IgG1. The diminished induction of transcription observed for these genes in the presence of α -IFNAR1 was striking, as message levels were reduced by 68% for *Cxcl9*, 70% for *Ilgp1*, and 81% for *Igtp*. Intriguingly, induction of *Oasl2* transcription was completely suppressed, as message levels in α -IFNAR1-treated mice were reduced to the baseline level obtained for uninfected C3H mice (Table I). Together, these data provide strong evidence that Type I IFN is involved in induction of the IFN-responsive profile at 1 week post-infection. The above results also strongly link Type I IFN with arthritis development and indicate that Type I IFN may partially exert its pro-arthritic effects through enhanced transcription of IFN-responsive genes by innate immune cells residing within ankle tissue. The kinetics of *B. burgdorferi* dissemination within α -IFNAR1-blocked mice was also monitored at 1 week post-infection to assess whether the spirochete burden within the rear ankle joints differed between these mice and those receiving IgG1 or PBS prior to infection. Quantitative RT-PCR utilizing primers specific for 16S rRNA revealed low but overlapping *B. burgdorferi* numbers within the joints of mice from all treatment groups at 1 week post-infection (data not shown), indicating that administration of α -IFNAR1 did not alter bacterial numbers in the joints.

IFN γ blockage has no effect on arthritis development or host defense to *B. burgdorferi* but does suppress induction of IFN-responsive gene transcripts at 1 and 2 weeks post-infection

As an additional test of the selective effect of Type I IFN on arthritis development, we decided to inhibit Type II IFN. Mice were administered α -IFN γ blocking antibody, PBS, or hamster IgG (hIgG) at various intervals both prior to, and following *B. burgdorferi* infection. Blockage of IFN γ did not impact arthritis development in mice examined at 4 weeks post-infection (data not shown), consistent with a previous report that IFN γ -/- C3H mice develop severe Lyme arthritis similar to that noted in wild-type mice (17). The effect of IFN γ inhibition on *B. burgdorferi* numbers within ankle tissues at 4 weeks post-infection was assessed by qPCR and there were no significant differences in the number of spirochetes detected within the ankle joints of α -IFN γ treated mice, when compared with

values obtained for control treatment groups (data not shown). These data support Brown and colleagues' previous conclusion that control of spirochete numbers within the ankle joints of infected C3H mice is IFN γ -independent (17).

Although arthritis proceeded uninhibited in IFN γ -blocked mice, α -IFN γ -treated mice exhibited a striking 100% reduction in the upregulated expression of the IFN-responsive genes *Igtp* and *Cxcl9*, when compared with mice receiving PBS or hIgG at 1 week post-infection. *Iigp1* transcript levels were 97% suppressed in α -IFN γ -blocked mice (Table II). *Oasl2* gene induction can be mediated by either Type I IFN or IFN γ (47,48). Interestingly, IFN γ inhibition caused a 73% suppression of *Oasl2* (Table II). In light of the 100% reduction in *Oasl2* message levels obtained with the α -IFNAR1 blocking antibody (compare Table I and Table II), these data suggest that *Oasl2* induced expression is more effectively reduced by Type I IFN blockage. Our previous microarray study found that while expression of IFN-responsive genes declined by 2 weeks post-*B. burgdorferi* infection, message levels for many transcripts were still several-fold higher than those obtained for uninfected mice (15). For this reason, the effect of IFN γ blockage was also assessed at 2 weeks post-infection. Markedly reduced transcript levels (90–100% suppression) were obtained for *Igtp*, *Iigp1*, and *Cxcl9*, whereas α -IFN γ treatment had little effect on *Oasl2*, suppressing its expression by only 43%, when compared with PBS- and hIgG- treated mice (Table II). Analyses of 16S rRNA transcript levels indicated that similar, low levels of *B. burgdorferi* were present within the joints of mice from all treatment groups at both 1 and 2 weeks post-infection (data not shown), indicating that IFN γ blockage did not alter the kinetics of bacterial dissemination. Finally, we assessed the effect of simultaneous blockage of both Type I IFN and IFN γ on induction of IFN-responsive genes at 1 week post-infection. Induced expression of all four examined IFN-responsive transcripts was 100% abolished within the joints of C3H mice injected with both α -IFNAR1 and α -IFN γ , as message levels were suppressed below the baseline values obtained for untreated, uninfected mice (Table III). Together these results support the unique role of Type I IFN in arthritis development, but also provide compelling evidence that induction of IFN-responsive genes within the joints of C3H mice at 1 week post-infection reflects the contribution of both Type I and II IFNs.

Plasmacytoid (pDC) and conventional DC (cDC) traffic to the joints following infection, but are not the initial source of *B. burgdorferi*-induced Type I IFN

In an attempt to identify the cell type(s) responding to Type I IFN to trigger arthritis development, we next questioned whether dendritic cells, either resident in, or trafficking to the joint following infection, are involved in the production of Type I IFN and/or in Lyme arthritis development. pDCs have long been recognized as the major producers of, and responders to Type I IFN, but cDCs also possess the ability to both synthesize and react to Type I IFN (52,53). To this end, we assessed whether pDCs and/or cDCs traffic to the joint following *B. burgdorferi* infection. Cells released from collagenase-digested ankle joints excised from C3H mice at 7 and 14 days post-infection were analyzed for CD11c and B220 surface expression, and compared with values obtained from uninfected ankle joints. By 7 days post-infection an increase in the numbers of both CD11c+B220- (myeloid or cDCs) and CD11c+B220+ (pDCs) cells were observed in C3H ankle joints, with even greater numbers observed at 14 days post-infection (Fig. 4). However, blockage of pDCs in C3H mice utilizing α -PDCA-1 antibody had no impact on arthritis severity (data not shown). Taken together, these results demonstrate that infiltration of both cDCs and pDCs by 1 week post-infection may occur as a response to infection and inflammation, but that pDCs do not mediate arthritis development in C3H mice.

However, since increased numbers of DCs were detected in infected joint tissue as early as 1 week post-infection, a time-frame coincident with IFN-responsive gene induction, we also

investigated the ability of both splenic pDCs and bone marrow-derived cDCs to induce expression of the IFN-profile in response to *in vitro* *B. burgdorferi*-stimulation. Although both cDCs and pDCs exhibited elevated *IL-6* and/or *IL-12* transcripts and protein (data not shown) in the presence of live *B. burgdorferi*, IFN-responsive transcripts were not induced in response to bacterial stimulation alone. In addition, pDCs stimulated in the presence of IFN α and *B. burgdorferi* also failed to upregulate IFN-inducible genes. However, a synergistic increase in expression was detected in cDCs administered IFN β and live spirochetes, suggesting that priming may be required for optimal cDC contribution to the IFN response (Table IV). It has been reported that pDCs exhibit a diminished capacity for phagocytosis of antigens, relative to other APCs, such as cDCs and macrophages (52). To enhance the ability of pDCs to internalize live bacteria, antibody to the abundantly expressed outer surface protein A (OspA) was added to pDCs or cDCs, prior to the addition of *B. burgdorferi*. Unexpectedly, incubation of cDCs with α -OspA antibody resulted in induction of all four examined IFN-responsive genes, but not in *IL-12* or *IL-6* transcripts. In the presence of opsonizing antibody, neither *Borrelia*-stimulated pDCs nor cDCs exhibited further upregulation of IFN-responsive genes (Table IV). Taken together, the above data strongly imply that while DCs may contribute to either amplification of IFN-profile genes, or resolution of this response *in vivo*, they are unlikely to be the first cell type driving the transcription of Type I IFN-responsive genes in response to *B. burgdorferi* infection.

Bone marrow-derived macrophage induction of IFN-responsive genes in response to *in vitro* *B. burgdorferi*-stimulation is Type I IFN-dependent and occurs via a MyD88-independent signaling pathway

Another innate immune candidate cell, the bone marrow-derived macrophage (BMM ϕ) was then evaluated for its ability to upregulate IFN-inducible genes in response to *B. burgdorferi*. Twenty-four hours after the addition of *B. burgdorferi*, transcripts for *Igtp* (Fig. 5A), *Iigp1* (Fig. 5B), *Oasl2* (Fig. 5C), and *Cxcl9* (Fig. 5D) were upregulated in BMM ϕ isolated from both C57BL/6 and C3H/HeN mice. BMM ϕ from C3H/HeJ mice, which lack a functional *Tlr4* gene (54), also displayed upregulation of IFN-responsive transcripts, indicating that transcription of these genes was not due to low-level LPS contamination of our *B. burgdorferi* grown in complex media (Fig. 5). To assess whether borrelial lipoproteins or unmethylated CpG DNA were being sensed by TLRs 2 and 9, respectively, to enact transcription of IFN-inducible genes, BMM ϕ isolated from TLR2 $^{-/-}$ and TLR9 $^{-/-}$ mice were stimulated with *B. burgdorferi*. Transcription of IFN-inducible genes proceeded in the absence of TLR 2 or 9, indicating that these TLRs are not utilized by *B. burgdorferi* to upregulate IFN-responsive transcripts. Induction of IFN-responsive transcripts is also MyD88-independent, as MyD88 $^{-/-}$ BMM ϕ stimulated with *B. burgdorferi* upregulate IFN profile genes. Intriguingly, BMM ϕ isolated from IFNAR1 $^{-/-}$ mice failed to induce IFN-responsive genes as a result of *B. burgdorferi* stimulation. This observation provides strong evidence that Type I IFN modulates transcription of IFN-responsive genes in macrophages, and underscores the utility of the macrophage as a model cell to examine the mechanisms of IFN-responsive gene induction following *B. burgdorferi* infection.

NK cell depletion has no effect on arthritis development or host defense to *B. burgdorferi* at 4 weeks post-infection, but NK cells are major IFN γ contributors to induction of the IFN-responsive profile at 1 week post-infection

Our above results demonstrating that IFN γ blockage suppressed IFN-responsive gene transcription without impacting arthritis severity was intriguing (Table I), especially in light of conflicting data in the field concerning a role for IFN γ in Lyme arthritis development (17,50,51). Our finding that IFN-responsive genes are still induced in C3H *scid* mice (Fig. 1) indicated that cells other than T lymphocytes were responsible for both the Type I- and IFN γ -inducible portions of the profile. NK cells are potent producers of IFN γ , and have been

previously shown to secrete this cytokine in response to *B. burgdorferi* (55) (56). NK cells were depleted from C3H mice using α -asialo GM1, to determine whether these cells impact arthritis development or host defense to *B. burgdorferi*. α -asialo GM1 was employed to neutralize NK cells because C3H mice produce the NK1.2 allele, to which there are no commercially available antibodies. In agreement with data previously obtained with the footpad-injection model (56), ankle swelling measurements and histopathological lesion scores obtained for α -asialo GM1-injected mice did not differ significantly from those recorded for PBS- or vehicle-treated animals (data not shown). Similar numbers of spirochetes were present in both the joints of α -asialo GM1-treated mice and in control mice at 4 weeks post-infection (Table V), indicating that NK cell depletion does not affect the ability of the host immune system to respond to *B. burgdorferi* challenge. We also examined whether NK cells are a major source of IFN γ driving the induction of the IFN-profile at 1 week post-*B. burgdorferi*-infection. Strikingly, mice treated with α -asialo GM1 exhibited a significant reduction (77–98%) in the expression levels of *Igtp*, *ligp1*, and *Cxcl9*, when compared with message levels obtained for PBS- and rabbit IgG-treated mice. In contrast, *Oasl2* message levels were only decreased by 54% within the joints of α -asialo GM1 treated mice (Table V), an observation that supports other data presented in this report indicating that the magnitude of *Oasl2* expression shows greater dependence on Type I IFN. These data clearly demonstrate that NK cells are a major IFN γ source contributing to induction of IFN-responsive profile genes at 1 week post-infection. However, as we have reported here, in confirmation of previously published reports (17,56), NK cells and IFN γ production do not contribute to arthritis development, reinforcing the idea that the entire IFN-profile is not required for arthritis development.

Discussion

Distinct severities of Lyme arthritis are found in different strains of inbred mice, providing an opportunity to compare responses to *B. burgdorferi* infection in joint tissue of mice developing mild versus severe disease. Affymetrix microarray analyses previously conducted in our laboratory demonstrated that a large number of IFN-responsive genes were strongly upregulated within the joints of C3H mice by 1 week post-*B. burgdorferi* infection. This gene induction profile was specific to the joints of mice destined to develop severe arthritis, and was not found in other tissues of these mice (spleen, ear). Because the genes of this profile are annotated in the literature as being either Type I IFN-responsive, IFN γ -responsive, or both, it was not clear which IFN was governing their induction (15). Therefore, in this report antibodies that block the Type I IFN receptor or IFN γ were used to determine which of these IFN pathways were responsible for the observed gene expression profile, and if either was involved in arthritis development. Blockage of IFNAR1 prior to infection with *B. burgdorferi* was uniquely associated with a reduction in arthritis severity, whereas, inhibition of IFN γ did not effect arthritis (Fig 2,3). Neither signaling pathway was implicated in control of *B. burgdorferi* in tissues (Fig. 2, Table V). The partial inhibition of arthritis by α -IFNAR1 is consistent with Quantitative Trait Loci (QTL) analysis, which has identified at least 6 QTL that are responsible for the greater arthritis severity in C3H mice than in C57BL/6 mice (57,58). This clearly supports the concept that additional signaling pathways not influenced by Type I IFN continue to contribute to arthritis severity in α -IFNAR1 antibody-blocked mice. Our data differ from two 1995 reports that found a decrease in arthritis severity and spirochete cultivation following α -IFN γ administration (50,51). Although it is difficult to explain this discrepancy, there are significant differences in the infection and arthritis protocols used previously and the more quantitative measures utilized in the current study. Importantly, our results are in agreement with more recent reports using gene ablations for IFN γ , which demonstrated that this IFN was not required for arthritis development, nor for the control of spirochete numbers within murine joint tissue (17,59).

Interestingly, blockage of either Type I IFN or Type II IFN signaling pathways caused a significant reduction in several of the hallmark transcripts of the C3H IFN profile at 1 week of infection (Table I, II, III). Although Type I IFN signaling can result in the phosphorylation and aggregation of different combinations of Stat molecules than those activated by IFN γ (60), and Stat1-independent signaling pathways have been identified for IFN γ (61,62), overlap between the Type I and II IFN signaling pathways has been well-documented in the literature. Both classes of IFNs trigger the Jak-Stat pathway during their activation, and share the ability to activate the transcription factors Stat1 and IRF1 (63). An additional layer of synergism also exists between Type I and II IFN signaling pathways, as Type I IFN induction results in downstream activation of the ISFG3 (Stat1: Stat2: IRF9) complex, and IFN γ production increases IRF9 levels (64). Even so, there was some selectivity as blocking Type I IFN signaling had a greater effect on induction of *oasl2* gene transcripts than did blocking of Type II IFN. Transcripts for other genes, *igtp*, *iigp* and *cxcl9*, were more dramatically reduced by IFN γ neutralization. These results indicate that the robust upregulation of all IFN inducible genes is not required for severe arthritis development, as many were suppressed by α -IFN γ without an effect on arthritis. In support of this idea, a previous study conducted on C3H Stat1 $^{-/-}$ mice indicated that Stat1 was dispensable for arthritis development, although exacerbated carditis was observed relative to wild-type mice following *B. burgdorferi* inoculation into the hind foot-pad (65). However, the more complete reduction of *oasl2* message by α -IFNAR1, along with its suppressive effect on arthritis development, suggests a skewed effect towards genes with IFN-stimulated response elements (ISRE) that are classically considered downstream of Type I IFN signaling. Thus, these results provide the first evidence linking early induction of Type I IFN to the development of severe Lyme arthritis.

Following infection with certain types of viruses Type I IFN produced by fibroblasts and cDCs triggers an IRF-7-dependent feedback self-amplification loop, resulting in the synthesis of massive quantities of IFN α/β by the cell (60). Blockage of Type I IFN in C3H mice with α -IFNAR1 could choke off this autocrine pathway of IFN α/β production, resulting in concomitant decreases in inflammation and arthritis severity. Results from infected joint tissue provided a snap shot of the evolution of disease, but did not provide insight on the cell types responsible for initiating and amplifying the IFN responses to infection. The observation that C3H *scid* mice, which develop severe arthritis, also display the IFN-responsive gene profile at 1 wk post-infection, indicated that neither T nor B lymphocytes were required for induction of these genes (Fig. 1). This finding indicated that transcription of IFN-responsive profile genes is mediated by cells of the innate immune system. Depletion of NK cells had no effect on arthritis development, but did cause reduction in the same transcripts that were suppressed by α -IFN γ (Table V). Importantly, *oasl2* transcript levels were less dramatically influenced by NK cell depletion than those considered downstream of IFN γ . This result implicates NK cells in the IFN γ response, while suggesting other cell types are the source of Type I IFN linked to arthritis development.

The known involvement of pDCs in production of Type I IFN in viral and in pathological responses prompted an assessment of the presence of these cells in the joint tissue of infected C3H mice. Both pDCs and cDCs infiltrate into infected joint tissue by 1 wk post-infection, with greater levels seen at 2 wks following infection (Fig. 4). However, pDCs cultured *in vitro* with *B. burgdorferi* did not generate transcripts of the IFN profile, although induction of IL-12 was demonstrated by both RT-PCR and ELISA analyses (Table IV and data not shown). In contrast, cDCs were able to transcriptionally upregulate components of the profile in response to *B. burgdorferi*; however, this required the addition of exogenous Type I IFN. While the above results suggest that DCs may contribute to the amplification of the IFN profile in infected C3H mice, DCs are not likely to be the first responding cell that initiates this unusual response. Although it is possible that primed cDCs may upregulate IFN

inducible transcripts, it seems likely that there is another cell type responsible for the initiation of the type I IFN production following infection.

Type I IFN is readily induced in bone marrow-derived macrophages following infection with a virus or intracellular bacteria (66). In contrast, little is known about elicitation of Type I IFN production in these cells upon extracellular bacterial stimulation. For this reason, we decided to assess whether *B. burgdorferi*-stimulated macrophages could induce IFN-responsive gene transcripts. BMM ϕ incubated with live *B. burgdorferi* readily induced *Igtp*, *Iigp1*, and all other examined IFN-responsive transcripts. Expression of these genes was entirely dependent on Type I IFN, as transcripts were completely suppressed in IFNAR1 $^{-/-}$ macrophages (Fig. 5). This observation strongly suggests that Type I IFN drives the transcription of IFN-responsive genes in macrophages, and emphasizes the usefulness of the macrophage as a model cell to investigate the pathways utilized by *B. burgdorferi* to stimulate expression of these genes. Induction of IFN-responsive genes by macrophages, and other myeloid cells, could heavily contribute to the severely arthritic phenotype exhibited by C3H mice, a scenario which gains credence from the observation that both MyD88 $^{-/-}$ and TLR2 $^{-/-}$ mice induce IFN-responsive genes and develop severe arthritis following *B. burgdorferi* infection (11–14). Macrophages have also been implicated in the development of rheumatoid arthritis, as has been demonstrated by experiments utilizing the K/B X N serum-transfer model (67). Experiments conducted with *B. burgdorferi*-stimulated BMM ϕ derived from TLR2 $^{-/-}$, TLR9 $^{-/-}$, and MyD88 $^{-/-}$ mice indicate that a MyD88-independent pathway is being utilized by the Lyme disease spirochete to induce the IFN-profile in these cells.

Results obtained with bone marrow-derived macrophages clearly demonstrate the existence of a new pathway by which *B. burgdorferi* induces Type I IFN, and its absence from IFNAR1 $^{-/-}$ macrophages indicates that Type I IFN is directly responsible for the downstream induction of IFN-responsive genes. The finding that macrophages from TLR2 $^{-/-}$ mice upregulate IFN inducible genes when incubated with *B. burgdorferi* are further illuminating, as there is no recognized signaling pathway emanating from TLR2 to Type I IFN. Our results provide compelling evidence for the presence of a sensor for *B. burgdorferi* in macrophages that results in the production of Type I IFN. Furthermore, the *B. burgdorferi* ligand for this receptor appears to be distinct from those previously characterized as activating the TLR2/MyD88 pathway. Others have found that phagocytosis of spirochetes enhances the induction of some pro-inflammatory cytokines (55,68), therefore, phagocyte mediated internalization may explain how this extracellular pathogen is able to intersect the upstream components of the Type I IFN signaling cascade. However, it has also been reported that MyD88 $^{-/-}$ macrophages are defective in the uptake of *B. burgdorferi*, suggesting that other mechanisms may be involved in induction of the IFN-profile (13,68). In support of the above-mentioned idea, it has recently been demonstrated that Group A *Streptococcus* (GAS), an extracellular bacterium that can survive but not replicate within macrophages, triggers the production of Type I IFN via a MyD88-independent pathway (69). Utilization of this pathway may be highly relevant for GAS pathogenesis, as these streptococci can also cause arthritis. Identification of this MyD88-independent receptor will undoubtedly shed light on the mechanisms utilized by *B. burgdorferi* to trigger induction of the IFN-profile. In conclusion, we now propose that an intracellular sensor for *B. burgdorferi* induces Type I IFN production and the downstream amplification of Type I IFN and IFN-inducible genes. Our results are of particular significance as we have demonstrated that this previously unrecognized signaling pathway is involved in Lyme arthritis development.

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Abbreviations

cDC	conventional dendritic cell
pDC	plasmacytoid dendritic cell
BMMϕ	bone marrow-derived macrophage
qPCR	quantitative polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
QTL	quantitative trait loci
ISRE	IFN-stimulated response element

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Figure 1. B and T lymphocytes do not contribute to induction of IFN-responsive genes
RT-PCR analysis of ankle joint RNA obtained from C3H *scid* mice at 1 week post-*B. burgdorferi* infection. *Igtp* (A), *ligp1* (B), *Oasl2* (C), and *Cxcl9* (D) transcripts were all elevated to similar levels, relative to uninfected animals, as those obtained for wild-type C3H mice. Transcript levels were normalized to 1000 copies of β -actin, and values shown represent the average $\pm$ standard deviations for 4 mice per group.

Figure 2. α-IFNAR1-treated C3H mice exhibit decreased arthritis severity at 4 wks post-B. burgdorferi infection, without impacting host defense

α-IFNAR1-treated C3H mice exhibit decreased ankle swelling (A), and histopathological lesion scores (B) relative to PBS- and/or IgG1-treated animals. $p < 0.05$ versus both control groups, as determined by one-way ANOVA with Bonferroni as the post-test in (A) and * $p < 0.05$ versus IgG1 (B), by Mann-Whitney U. (C) Spirochete numbers were detected within joint tissues analyzed from each antibody treatment group by qPCR using *RecA*, and normalized to the single-copy mouse gene *Nidogen*. Data shown are the averages $\pm$ standard deviations for 5 mice per group obtained from one experiment, and are representative of results obtained from two separate experiments.

Figure 3. Blockage of IFNAR1 suppresses many key histopathological features of Lyme arthritis Hematoxylin and eosin stained sections of rear ankle joints obtained from (A) Uninfected, or at 4 wks post-infection from (B) PBS-treated, (C) IgG1-treated, or (D) α -IFNAR1-treated mice were examined for key features of Lyme arthritis, including thickening of the cranial-tibial tendon sheath and neutrophil infiltration. While extensive tendon sheath thickening and neutrophil infiltration are evident in both the PBS (B) and IgG1-treated (C) mice, animals receiving α -IFNAR1 antibody (D) have significantly reduced tendon sheath thickening and neutrophil infiltration. All images were obtained using a 4X magnification. The arrow points to the cranial tibial tendon sheath. Data shown were obtained from one experiment, and are representative of results obtained from two separate experiments.

Figure 4. pDCs and cDCs traffic to *B. burgdorferi*-infected mouse joints as early as 7 days post-infection and are further elevated at 14 days post-infection

Collagenase released ankle cells obtained from three C3H mice at each time point were stained with CD11c and B220 and analyzed by flow cytometry. The numbers of CD11c⁺B220⁻ (cDC) and CD11c⁺B220⁺ (pDCs) were determined at each time point. For pDCs, * $p < 0.05$ at 14 days post-infection versus uninfected mice and for cDCs, * $p < 0.05$ at 14 days post-infection versus uninfected mice, by one-way ANOVA with Bonferroni as the post-test. Data shown are the averages + standard deviations obtained from one experiment, and are representative of results obtained from three separate experiments.

Figure 5. BMMφ isolated from both C3H and C57BL/6 mice induce IFN-responsive genes following *B. burgdorferi*-stimulation in a Type I IFN-dependent, MyD88-independent fashion RT-PCR was performed on BMMφ prepared as described in the materials and methods from the following strains of mice: C57BL/6, C3H/HeN, C3H/HeJ, C3H TLR2^{-/-}, B6 TLR9^{-/-}, B6 MyD88^{-/-}, and B6 IFNAR1^{-/-} mice. Transcript levels for *Igtp* (A), *ligp1* (B), *Oasl2* (C), and *Cxcl9* (D) were normalized to β -actin. Data shown are the averages $\pm$ standard deviations obtained from one experiment, and are representative of results obtained from three separate experiments.

Anti-IFNAR1 reduces IFN-inducible transcript expression in joint tissue of *B. burgdorferi*-infected C3H mice at 1 wk post-infection

Table 1

Pre-treatment of <i>B. burgdorferi</i> - infected mice	Transcript levels in joint tissue*			
	<i>Igtp</i>	<i>Igtp1</i>	<i>Oas12</i>	<i>Cxcl9</i>
PBS (5)	8.5 ± 6.1	65.4 ± 36.0	7.5 ± 4.0	101 ± 57.0
IgG1(5)	10.9 ± 9.7	84.3 ± 63.0	9.3 ± 5.0	157.0 ± 107.0
Anti-IFNAR1 (5)	2.7 ± 3.4 [†]	24.1 ± 20.0 [†]	1.6 ± 1.0 [†]	44.0 ± 25.0 [†]
Uninfected (16)	1.1 ± 0.4	3.0 ± 1.3	1.8 ± 0.9	5.3 ± 2.7
% Reduced by a-IFNAR1 [‡]	81	70	100	68

* Transcript levels determined by RT-PCR, normalized to 1000 copies of *β-actin*

[†] Significantly different from both PBS and IgG1, p < 0.05 by Mann-Whitney U test, except for *Igtp* transcript levels, which are significantly different from IgG1, p < 0.05 by Mann-Whitney U test

[‡] Average reduction seen versus PBS and IgG1 controls when compared with untreated, uninfected mice

Bold = *Oas12* expression is suppressed to the baseline level obtained for untreated, uninfected mice

Anti-IFN γ represses IFN-responsive transcripts in *B. burgdorferi*-infected joint tissue at 1 and 2 wks post-infection

Table II

Pre-treatment of <i>B. burgdorferi</i> -infected mice		Transcript levels in joint tissue at 1 wk post-infection *			
1 wk post-infection		<i>Igtp</i>	<i>Iigp1</i>	<i>Oasl2</i>	<i>Cxcl9</i>
PBS (5)		10.2 $\pm$ 6.5	57.9 $\pm$ 36.0	10.5 $\pm$ 4.7	46.8 $\pm$ 54.3
Hamster IgG (5)		14.9 $\pm$ 16.0	115.3 $\pm$ 99.2	14.6 $\pm$ 11.8	65.5 $\pm$ 44.8
Anti-IFN γ (5)		1.0 $\pm$ 0.8 [†]	5.0 $\pm$ 3.6 [†]	4.6 $\pm$ 2.2 [†]	2.9 $\pm$ 1.2 [†]
Uninfected (16)		1.1 $\pm$ 0.4	3.0 $\pm$ 1.3	1.8 $\pm$ 0.9	5.3 $\pm$ 2.7
% Reduced by a-IFN γ [‡]		100	97	73	100

Pre-treatment of <i>B. burgdorferi</i> -infected mice		Transcript levels in joint tissue at 2 wks post-infection *			
		<i>Igtp</i>	<i>Iigp</i>	<i>Oasl2</i>	<i>Cxcl9</i>
PBS (5)		5.0 $\pm$ 3.0	32.2 $\pm$ 13.3	4.1 $\pm$ 1.5	68.0 $\pm$ 43.3
Hamster IgG (5)		4.1 $\pm$ 1.0	27.6 $\pm$ 9.8	4.8 $\pm$ 2.2	54.4 $\pm$ 22.7
Anti-IFN γ (5)		1.1 $\pm$ 0.6 [†]	5.7 $\pm$ 3.1 [†]	3.3 $\pm$ 2.0	6.3 $\pm$ 2.0 [†]
Uninfected (16)		1.1 $\pm$ 0.4	3.0 $\pm$ 1.3	1.8 $\pm$ 0.9	5.3 $\pm$ 2.7
% Reduced by a-IFN γ [‡]		100	90	43	98

*Transcript levels determined by RT-PCR, normalized to 1000 copies of β -actin

[†]Significantly different from both PBS and isotype controls, p < 0.05 by Mann-Whitney U test

[‡]Average reduction seen versus PBS and IgG1 controls when compared with untreated, uninfected mice

Bold = % suppression of *Oasl2* expression is less than seen for other transcripts

Pre-treatment of *B. burgdorferi*-infected C3H mice with both anti-IFNAR1 and anti-IFN γ abolishes IFN-inducible transcript expression within joint tissue at 1 wk post-infection

Table III

Pre-treatment of <i>B. burgdorferi</i> -infected mice	Transcript levels in joint tissue*			
	<i>Igtp</i>	<i>Iigp1</i>	<i>Oasl2</i>	<i>Cxcl9</i>
PBS (5)	14.0 $\pm$ 8.1	63.7 $\pm$ 11.3	5.1 $\pm$ 0.8	80.7 $\pm$ 23.5
MOPC-21 + Hamster IgG (5)	10.8 $\pm$ 7.9	55.1 $\pm$ 28.9	6.3 $\pm$ 4.2	87.2 $\pm$ 58.0
Anti-IFNAR1 + Anti-IFN γ (5)	0.6 $\pm$ 0.3 [†]	1.0 $\pm$ 0.4 [†]	0.4 $\pm$ 0.3 [†]	1.7 $\pm$ 0.8 [†]
Uninfected (16)	1.1 $\pm$ 0.4	3.0 $\pm$ 1.3	1.8 $\pm$ 0.9	5.3 $\pm$ 2.7
% Reduced by α -IFNAR1 + α -IFN γ [‡]	100	100	100	100

* Transcript levels determined by RT-PCR, normalized to 1000 copies of β -actin

[†] Significantly different from both PBS and isotype controls, p < 0.05 by Mann-Whitney U test

[‡] Average reduction seen versus PBS and IgG1 controls when compared with untreated, uninfected mice

Table IV
Induction of IFN-responsive genes in plasmacytoid (pDC) and conventional (cDC) dendritic cells following B. burgdorferi (Bb) stimulation

Treatment (24 hours)	Transcript Levels*					
Plasmacytoid dendritic cells (pDC)	<i>Igtp</i>	<i>Iigp1</i>	<i>Oas12</i>	<i>Cxcl9</i>	<i>IL-12</i>	<i>IL-6</i>
Media	1.2 ± 0.6	3.7 ± 0.7	11.8 ± 3.4	0.2 ± 0.2	5.8 ± 3.2	0.0 ± 0.0
Bb (7.4 × 10 ⁶ /ml)	0.8 ± 0.0	1.4 ± 0.2	7.2 ± 0.2	0.2 ± 0.0	8.4 ± 0.7	0.5 ± 0.1
rIFNα (100 U/ml)	1.4 ± 0.1	6.3 ± 1.1	26.4 ± 0.2	0.0 ± 0.0	6.0 ± 0.1	0.0 ± 0.0
Bb + rIFNα	1.9 ± 0.4	4.2 ± 0.9	20.0 ± 0.6	0.2 ± 0.0	10.1 ± 0.2	0.5 ± 0.1
Anti-OspA (10 µg/ml)	0.8 ± 0.1	3.8 ± 0.7	12.1 ± 2.9	0.2 ± 0.3	4.0 ± 0.8	0.1 ± 0.0
Bb + anti-OspA	0.7 ± 0.3	1.5 ± 0.1	8.1 ± 0.7	0.2 ± 0.1	15.0 ± 10.1	0.4 ± 0.1
Conventional dendritic cells (cDC)						
Media	0.5 ± 0.0	0.1 ± 0.0	2.7 ± 0.6	0.1 ± 0.0	1.3 ± 0.2	0.0 ± 0.0
Bb (7.4 × 10 ⁶ /ml)	0.5 ± 0.0	0.0 ± 0.0	2.3 ± 0.4	0.0 ± 0.0	12.1 ± 3.4	8.6 ± 0.3
rIFNβ (1000 U/ml)	0.4 ± 0.1	0.0 ± 0.0	3.5 ± 1.2	0.1 ± 0.1	1.1 ± 0.1	0.2 ± 0.0
Bb + rIFNβ	9.7 ± 1.2	23.0 ± 0.2	44.7 ± 2.6	15.1 ± 4.3	23.0 ± 6.9	75.4 ± 9.9
Anti-OspA (10 µg/ml)	19.9 ± 0.9	58.5 ± 5.0	96.8 ± 19.6	14.8 ± 1.9	1.3 ± 0.1	0.0 ± 0.0
Bb + anti-OspA	17.8 ± 1.6	40.7 ± 1.9	76.5 ± 1.7	21.0 ± 2.8	5.4 ± 3.9	4.6 ± 2.9

* Transcript levels determined by RT-PCR, normalized to 1000 copies of *β-actin*

Depletion of NK cells with anti-asialo GM1 represses IFN-inducible transcripts within the rear ankle joints of C3H mice at 1 wk post-B. burgdorferi (Bb) infection, but does not impact host defense at 4 weeks post-infection

Table V

Pre-treatment of <i>B. burgdorferi</i> -infected mice	Transcript levels in joint tissue at 1 wk post-infection*				Bb#s within joint tissue at 4 wks post-infection#
	<i>Igtp</i>	<i>Iigp1</i>	<i>Oasl2</i>	<i>Cxcl9</i>	
PBS (5)	33.3 ± 27.9	115.3 ± 72.1	18.8 ± 6.1	170.9 ± 107.7	16 ± 9
Rabbit IgG (5)	33.2 ± 21.4	109.4 ± 41.5	25.1 ± 6.4	71.4 ± 27.4	23 ± 20
Anti-asialo GM1 (5)	7.4 ± 4.2 [†]	26.7 ± 14.6 [†]	10.9 ± 6.5 [†]	17.9 ± 11.6 [†]	26 ± 21
Uninfected (16)	1.1 ± 0.4	3.0 ± 1.3	1.8 ± 0.9	5.3 ± 2.7	0 ± 0
% Reduced by a-asialo Gm1 [‡]	98	77	54	87	NA

* Transcript levels determined by RT-PCR, normalized to 1000 copies of *β-actin*

[†] Significantly different from both PBS and isotype controls, $p < 0.05$ by Mann-Whitney U test

[‡] Average reduction seen versus PBS and IgG1 controls when compared with untreated, uninfected mice

Bold = % suppression of *Oasl2* expression is less than seen for other transcripts

The number of *B. burgdorferi* genomes within the rear ankle joints was determined by quantitative PCR and normalized to 1000 copies of the mouse *nidogen* gene.

NA = Not applicable