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CD28 Deficiency Exacerbates Joint Inflammation upon Borrelia burgdorferi Infection, Resulting in the Development of Chronic Lyme Arthritis

Bettina P. Iliopoulou,* Joseph Alroy,† and Brigitte T. Huber2*

Lyme disease, caused by the tick-borne spirochete Borrelia burgdorferi (Bb), is a multisystem illness, affecting many organs, such as the heart, the nervous system, and the joints. Months after Bb infection, ~60% of patients experience intermittent arthritic attacks, a condition that in some individuals progresses to chronic joint inflammation. Although mice develop acute arthritis in response to Bb infection, the joint inflammation clears after 2 wk, despite continuous infection, only very rarely presenting with chronic Lyme arthritis. Thus, the lack of an animal system has so far prevented the elucidation of this persistent inflammatory process that occurs in humans. In this study, we report that the majority of Bb-infected CD28−/− mice develop chronic Lyme arthritis. Consistent with observations in chronic Lyme arthritis patients, the infected mutant, but not wild-type mice present recurring monoarticular arthritis over an extended time period, as well as anti-outer surface protein A of Bb serum titers. Furthermore, we demonstrate that anti-outer surface protein A Abs develop in these mice only after establishment of chronic Lyme arthritis. Thus, the Bb-infected CD28−/− mice provide a murine model for studying chronic Lyme arthritis. *The Journal of Immunology, 2007, 179: 8076–8082.

Lyme disease is the most common tick-borne illness in the United States, with >20,000 cases reported per year (1). The clinical manifestations of this disease are initiated upon transmission of the spirochetal bacterium Borrelia burgdorferi (Bb),3 the causative agent of Lyme disease, into the mammalian host by an infected tick of the Ixodes scapularis family. After dissemination in the skin, Bb seems to localize mainly to the heart, the nervous system and the joints (2), eliciting an inflammatory response in these organs. A strong innate immune reaction against Bb develops in the joints leading to an adaptive inflammatory Th1 response (3, 4), a condition that affects 60% of untreated Bb infected individuals. These Lyme arthritis patients present recurring, monoarticular arthritic attacks, mainly in the knee that may persist for years (5). Although a number of studies have attempted to elucidate the mechanisms by which Lyme arthritis is induced in humans, there is still much to learn, in particular about the role of the adaptive immune system in the remission and the aggravation of the disease.

Wild-type (wt) mouse strains, as well as mice carrying certain cytokine or T and B cell deficiencies have been employed to dissect the immunological events leading to joint inflammation upon Bb infection (6–10). It is well established that the development and severity of Lyme arthritis in the murine system upon Bb needle inoculation is strain, age, and dose dependent (11, 12). Studies in SCID mice revealed that joint inflammation is exacerbated in the absence of an adaptive immune response, mainly due to unopposed expansion of the pathogen burden (7, 10, 13). In contrast, it has been suggested that in immunocompetent mice CD4+ Th1 cells contribute to the exacerbation of Lyme arthritis, as well as to the control of spirochetes, allowing the development of an armistice where the inflammatory response is ablated, despite persistent infection. Murine Lyme arthritis peaks within the first 2 wk post infection and then resolves spontaneously and only very few individual mice continue to present chronic arthritis (14). Thus, it has not been possible so far to use the murine system for the analysis of the immunological events leading to chronic Lyme arthritis.

It seems that in the murine system persistent Bb infection is well contained, resulting in the absence of an inflammatory response. Excessive active immune response leads to immunopathology that can be detrimental for the host. To avoid this, the immune system has developed mechanisms to be “tolerized” in the presence of the spirochetes. This “tolerance” is being maintained by immunoregulatory mechanisms and is being abrogated in patients that present chronic Lyme arthritis. Immunoregulation is mediated by various factors, such as CTLA-4, IL-10, and CD4+CD25+ Treg cells. Thus, we hypothesized that by diminishing these immunomodulatory effects, we may increase the incidence of chronic Lyme arthritis in mice.

In search of a murine model for chronic Lyme arthritis, we decided to employ the CD28−/− mouse for our studies. CD28 is a major costimulatory molecule that mediates efficient T cell activation. Nevertheless, infection of CD28−/− mice with various pathogens leads to a potent Ag specific T cell response (15–17). This mouse presents a unique possibility: on the one hand, its ability to mount an active immune response to pathogen is unaffected; on the other hand, one of the main immunoregulatory factors, CTLA-4, is impaired, resulting in chronic arthritis.

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3 Abbreviations used in this paper: Bb, Borrelia burgdorferi; OspA, outer surface protein A; Treg, regulatory T cells; wt, wild type; TRLA, treatment-resistant Lyme arthritis; AUC, area under the curve.

The Journal of Immunology
mechanisms, the presence of CD4+CD25+ regulatory T cells (Treg), is being compromised. Recent studies have reported an essential role of the CD28 costimulatory molecule in the development and maintenance of CD4+CD25+ Treg cells and have documented a dramatic reduction (80%) of these cells in the CD28−/− mice (18).

When the role of the B7/CD28 costimulatory pathway was addressed in the development of the acute murine Lyme arthritis by CD80/CD86 blockade, no difference in arthritis severity was observed between treated and untreated BALB/c mice (19). In the same study, the authors reported expansion of IFN-γ-producing T cells upon Bb infection. These data collectively led to the hypothesis that in the Bb infectious system the CD28 molecule is not required for the induction of an immune response against the pathogen, and that the signal for proper T cell activation could be provided by other costimulatory pathways. In the current study, we demonstrate that even though there is no difference in acute arthritis development between the Bb−infected CD28−/− and the control mice, CD28−/− mice develop higher incidence and severity of arthritis at later time points of disease progression, providing a murine model to investigate further the mechanism that regulates chronic Lyme arthritis.

Materials and Methods

Mice

Male CD28−/− mice on the C57BL/6J (B6) background were bred at the Tufts University Division of Laboratory Animal Medicine from breeding pairs that were initially obtained from The Jackson Laboratory. Age- and sex-matched wt B6 mice were purchased. All animal experiments were approved by IACUC, the institutional animal review board at Tufts-New England Medical Center. Four- to five-week-old male CD28−/− mice and wt mice were infected intradermally in the skin of the cubital area of both hind limbs (1 cm above the knee) with a total dose of 2 × 10^6 Bb per mouse. This protocol was used for all the infections.

B. burgdorferi

Low passage (passage 2) of the infectious Bb N40 clone D10E9A1-E (gift from Jennifer Coburn) (20, 21) was used for all infections. Bb were cultured in complete Barbour-Stoenner-Kelly medium (Sigma-Aldrich) at 34°C until mid-log phase (5 × 10^7 Bb/ml) and were counted by darkfield microscopy.

Histopathology

Ankles were harvested and decalcified overnight in Decalcer I solution (Sur-gipath). Next day, they were rinsed with distilled H2O for 30 min and then fixed in formalin until they were further processed. Hearts were cut in half and kept in formalin. Fixed ankles and heart tissues were embedded in paraffin, sectioned and stained by H&E. Stained sections were scored histopathologically in a blinded fashion on a scale from 0 to 3, based on the amount of infiltrating cells and morphology, as previously described (22): 0, no inflammation (no infiltrating cells were observed); 1, mild inflammation (one or several small and discrete areas of infiltrate); 2, moderate inflammation (multiple infiltrates without other gross changes); or 3, severe inflammation (heavy contiguous infiltrates in joints, tendons, and muscle).

Bb burden

DNA was extracted from ear punch tissue as previously described (23), with the following modifications. The ear punch tissue was digested for 4 h at 37°C in 125 μl of 1 mg/ml collagenase A (Roche) in Dulbecco’s PBS without Ca^2+/Mg^2+ (Cellgro), followed by overnight incubation at 55°C of 0.8 mg/ml Protease K (Invitrogen) in 125 μl of 100 mM Tris-HCl (pH 8.8), 5 mM EDTA, 0.2% SDS, and 200 mM NaCl. DNA was then extracted by 250 μl of buffer-saturated phenol (Invitrogen), then by an equal volume of phenol-chloroform-isooamyl alcohol 25:24:1 (Invitrogen) and last by an equal volume of chloroform. DNA was then precipitated with 0.3 M NaAc (pH 5.5) in 100% ethanol and resuspended in PCR quality H2O (Invitrogen) and used as template for determination of Bb burden. The Bb burden is determined by real-time qPCR. Specific primers and 6-carboxyfluorescein-labeled TaqMan MGB probes for Bb RecA (GenBank accession no. U23457) were designed and used for Bb detection (F-TGGGATAGCTGCTTTTATTGATGCT, R-GACTAAGCCAAAAGTTCTGCAACATT, P-CCTGTtTTATGCAAAGCTT), whereas primers and VIC-labeled probes specific for murine 18S were purchased (no. 4319431E, Applied Biosystems) and used as loading control. The PCR was conducted using iTaq SuperMix with Rox (Bio-Rad) per the manufacturer instructions. In brief, 100 ng of extracted DNA was used as template in 20 μl of reaction containing primers and probes specific for RecA and 18S (primers at 900 nM each, TaqMan MGB probes at 250 nM each). A Bb standard curve was generated by serial dilutions (1/10) of Bb genomic DNA of known concentration. Based on the calculation that 1 Bb genome is ~1.62 × 10^9 μg, the Bb standard curve spans from 1.5 × 10^7 to 1.5 Bb in 100 μg of mouse genomic DNA. The qPCR was performed on a 7300 real-time PCR system (Applied Biosystems), using the following PCR cycling parameters (95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min). Data were analyzed per manufacturer’s instructions. The amount of template DNA was first normalized by the signal of the 18S housekeeping gene. The normalized RecA signal was then used to determine the Bb burden, based on the standard curve.

Anti-Bb and anti-OspA ELISA

Flat bottom Immunol 2HB plates (Fisher Scientific) were coated overnight with 10 μg/ml Bb lysate or 5 μg/ml recombinant OspA (0.1 M Na2HPO4 (pH 9)). Uncoated wells were served as non-Ag control. Coated and uncoated wells were then washed with PBS/0.05% Tween 20 and blocked in 1% milk for 45 min at 37°C. Serum samples were diluted in 1% milk 1/400

![FIGURE 1. CD28−/− develop intermittent arthritis upon infection with Bb. CD28−/− (n = 5) and wt mice (n = 5) were infected with 2 × 10^6 Bb/mouse. Mock-infected mice (n = 2) are represented by the open squares. Arthritis was assessed periodically by measuring the ankles using a caliper. We measured both ankles on all mice and averaged the values. Error bars, SEM. Asterisks indicate significant difference in ankle swelling between the CD28−/− and the B6 mice, *p < 0.01; Student’s two-tailed, unpaired equal variance t test. Shaded areas represent episodes of recurring arthritis in CD28−/− mice. Statistical significance has been also confirmed based on the AUC for each group (p = 0.05). Data correspond to one experiment, representative of six independent experiments.](http://www.jimmunol.org/lookup/fig/1/11005/H11005)
for the anti-Bb ELISA or 1/100 for the OspA ELISA. Serially 2-fold dilutions of a known concentration of an OspA-specific mAb (LA-2) were also plated on each plate as a standard curve for the OspA ELISA. Samples were added in duplicates to the ELISA plates and incubated for 45 min at 37°C. Plates were washed and incubated with alkaline phosphatase (AP)-conjugated anti-mouse κ or anti-mouse IgM-AP, IgG3-AP, IgG1-AP, IgG2b-AP, IgG2c-AP (Southern Biotech) for 1 h at 37°C. We assess the levels of IgG2c, because B6 mice lack the gene for IgG2a and express instead the IgG2c isotype. Plates were then developed with the AP substrate, para-nitrophenyl phosphate (Pierce), in 0.2% diethanolamine substrate buffer (Pierce), and were read at 405 nm in a SpectraMax spectrophotometer (Molecular Devices).

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism software. All data were tested for Gaussian distribution, using the Shapiro-Will normality test. Quantitative differences between groups were assessed by a two-tailed Student’s t test (if two groups are compared), and ANOVA (if more than two groups are compared) for normally distributed data and Mann-Whitney U test (if two groups are compared) and Kruskal-Wallis test (if more than two groups are compared) for skewed data. Statistical differences in the proportion of mice with anti-OspA Abs were determined by a two-tailed Fisher’s exact probability test. For outcomes measured multiple times we compared differences between time points using area under the curve (AUC). Significance was declared at a two-sided 0.05 level for all statistical analyses.

**Results**

**CD28−/− mice develop recurring chronic arthritis upon Bb infection**

To investigate the role of the CD28 costimulatory molecule in the development of chronic Lyme arthritis upon Bb infection, CD28−/− and wt B6 mice were infected with a total dose of 2 × 10^4 Bb per mouse, as described in the Materials and Methods section. Arthritis was assessed in a blinded fashion two to three times a week by measuring the anterior-posterior tibiotarsal joint thickness using a caliper. This allows monitoring the edema formation over the course of the infection. The arthritic manifestations in Bb-infected mice were recorded over a period of 5 mo post infection. As seen in Fig. 1, there was no difference in arthritis development between the CD28−/− and the control mice at the early stage of the disease, implying that the ability of the CD28−/− mice to mount an innate and adaptive immune response against Bb is not compromised. However, while the majority of the B6 control mice resolved joint inflammation by 3–4 wk post infection, CD28−/− mice continued to develop recurring arthritic attacks, similarly to what had been observed in untreated Lyme arthritis patients (Fig. 1). The proportion of CD28−/− mice that developed chronic Lyme arthritis over time was statistically significant, as compared with the wt B6 mice (Table I). These episodes of arthritis were brief, lasting ~2 wk, followed by a remission phase.

**FIGURE 2.** CD28−/− develop monoarticular arthritis upon infection with Bb. In this graph, each mouse ankle is represented by a different color. Left (L) and right (R) ankles of wt mice (n = 5) (a) and left and right ankles of CD28−/− mice (n = 5) (b). During the late stage of the disease, the intermittent arthritic attacks seen in CD28−/− mice are in most cases monoarticular. Data correspond to one experiment, representative of six independent experiments.

**FIGURE 3.** Increased Lyme arthritis severity in CD28−/− mice. A, Histopathological scoring of arthritis severity in Bb-infected CD28−/− (n = 5) and wt mice (n = 5). At day 160 post infection, mice were sacrificed, and ankles were processed for histology. Arthritis severity was scored on a scale from 0 (no infiltrates or changes) to 3 (severe disease). Each symbol represents an individual ankle and the horizontal line represents the mean. The asterisk indicates significant difference, *p = 0.0003; Student’s two-tailed, unpaired equal variance t test. Mock-infected control mice exhibited normal histology. B, Scatter plot of ankle width vs arthritis severity score in CD28−/− mice. A significant correlation was found between ankle width and arthritis severity score, by linear correlation two-tailed analysis, p = 0.013 (Spearman correlation coefficient = 0.76). Data correspond to one experiment, representative of three independent experiments.

**Table I. CD28−/− B6 mice develop chronic Lyme arthritis upon infection with Bb**

<table>
<thead>
<tr>
<th>Group</th>
<th>Arthritis (arthritic mice/total mice)</th>
<th>Ankle Width (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28−/−</td>
<td>25/28*</td>
<td>3.2 ± 0.14</td>
</tr>
<tr>
<td>B6</td>
<td>7/29</td>
<td>2.9 ± 0.21</td>
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*p CD28−/− (n = 28) and wt B6 mice (n = 29) were infected with 2 × 10^4 Bb/mouse. Arthritis was assessed periodically by measuring the ankles using a caliper. The number of mice that develop chronic Lyme arthritis either in one (monoarticular) or both ankles is shown. Data include the pooled results of six independent experiments. *p = 7 × 10^-7 (two-tailed Fisher exact probability test).
Interestingly, the arthritic attacks during the later stage of the disease were mostly monoarticular, as seen in a more detailed graphic representation of each ankle (Fig. 2), again consistent with the clinical observations in Lyme patients. Thus, the CD28 deficiency in Bb-infected B6 mice prevents resolution of edema, as measured by joint swelling, leading to a perpetuated joint inflammation that appears to be in most cases recurring and monoarticular.

CD28−/− mice develop increased arthritis severity

To assess cellular infiltration in the inflamed joints, mice were sacrificed 5 mo post infection, during the third recurring arthritic episode. The tibiotarsal joints were processed for histology; slides were stained with H&E and were scored blindly for arthritis severity on a scale of 0 (no inflammation) to 3 (severe cellular infiltration). As shown in Fig. 3A, histological analysis showed increased arthritis severity and joint degeneration, a feature of chronically inflamed joints, in CD28−/− infected mice compared with the control group (p = 0.0003). Since in most cases the observed arthritis was monoarticular, we investigated whether the edema formation measured by the caliper correlated with cellular infiltration in each joint. As seen in Fig. 3B, there was a direct correlation between arthritis severity and ankle thickness (Spearman correlation coefficient = 0.76; p = 0.013).

CD28−/− mice control Bb burden similarly to wt mice

Increased arthritis in the Bb-infected CD28−/− mice may reflect the inability of these mice to control the spirochetes. To address this possibility, we compared the systemic Bb burden in these mice and wt controls. DNA was isolated from the ear punches of the infected mice during the chronic phase of Lyme arthritis and tested by real-time qPCR for the level of the Bb-specific gene, RecA. As can be seen in Fig. 4, there was no difference in RecA between the CD28−/− and the control group, indicating that the chronic immunopathology observed in the CD28−/− mice is not due to the inability of these mice to kill Bb.

CD28−/− mice develop anti-OspA Abs that correlate with the establishment of chronic Lyme arthritis

To delineate the humoral response in the Bb-infected CD28−/− mice, we bled the mice periodically and assessed the anti-Bb serum levels. Since Lyme arthritis patients develop anti-OspA IgG titers late in the course of the disease (24, 25), we measured the anti-OspA Ab levels in these mice as well. As shown in Fig. 5A, by day 200 post infection the CD28−/− B6 mice developed lower anti-Bb Ab levels compared with the control group (p = 0.001). Surprisingly, however, the humoral response to OspA did not follow the same pattern. Although B6 control mice had no detectable levels of anti-OspA Abs, CD28−/− mice developed substantial anti-OspA
A decrease in IgG1, IgG2a, and IgG2b isotypes and an increase in mice/total mice (A) and arthritic numbers represent mice with anti-OspA Ab/total mice (B). Developed anti-OspA Ab mainly of the IgM, IgG3, and IgG2b isotype (Fig. 5).

To further investigate the humoral response in these mice, we determined the isotype of the Bb-, as well as the OspA-specific Abs. As shown in Fig. 5, C and D, the pattern of the various Bb-specific isotypes was the same between the CD28−/− mice and control mice, with an overall decrease in IgG1, IgG2a, and IgG2b isotypes and an increase in IgM and IgG3 isotypes. We also observed that the CD28−/− mice developed anti-OspA Ab mainly of the IgM, IgG3, and IgG2b isotype (Fig. 5E).

Ab titers during the late stage of the disease, which coincides with the prolonged episodes of arthritis (Fig. 5B). To further investigate the humoral response in these mice, we determined the isotype of the Bb-, as well as the OspA-specific Abs. As shown in Fig. 5, C and D, the pattern of the various Bb-specific isotypes was the same between the CD28−/− mice and control mice, with an overall decrease in IgG1, IgG2a, and IgG2b isotypes and an increase in IgM and IgG3 isotypes. We also observed that the CD28−/− mice developed anti-OspA Ab mainly of the IgM, IgG3, and IgG2b isotype (Fig. 5E).

Collectively, these results indicate that the humoral immune response to OspA upon Bb infection is unique for the CD28−/− system and is dependent on the establishment of chronic Lyme arthritis.

**Chronic Lyme arthritis in CD28−/− mice resolves upon antibiotic treatment**

Upon establishment of chronic Lyme arthritis, CD28−/− mice were treated with antibiotic, and the joint inflammation was followed over a period of 2 mo. As shown in Fig. 7, arthritis did not persist after antibiotic treatment, as assessed by monitoring edema formation using a caliper. In contrast, untreated Bb-infected CD28−/− mice never resolved joint inflammation spontaneously, and a representative experiment is shown on Fig. 1. In addition, no Bb DNA was detectable in the ear punch of the CD28−/− mice, after antibiotic treatment (data not shown). The results were confirmed by histological analysis of the joints, performed upon termination of the experiment. No cellular infiltration was observed in the joints of the antibiotic-treated CD28−/− mice (data not shown).

**Discussion**

One of the most prominent clinical manifestations of Lyme disease is the development of chronic Lyme arthritis. Months after Bb infection, ~60% of untreated Lyme patients experience intermittent arthritic attacks that may last for years. Affected individuals have persistent Bb infection, as measured by spirochetal DNA PCR in their joints (5, 26). However, the immune mechanisms that govern this inflammatory process have been elusive thus far, mainly because of the lack of an animal system for chronic Lyme arthritis. Mice develop acute arthritis in response to Bb infection; their joint inflammation peaks within the first few weeks post infection and then spontaneously resolves, even though Bb establishes life-long persistent infection. It is likely, therefore, that immunoregulatory mechanisms control excessive immune activation in mice, allowing perseverance of the spirochetes. According to this model, interference with immunoregulation would lead to...
chronic arthritis in mice, similar to what is seen in the majority of Bb-infected humans.

To test this working hypothesis, we analyzed arthritis in CD28<sup>−/−</sup> mice after infection with Bb, because these mutant mice have a substantial reduction in the number of T<sub>reg</sub> cells (18). No difference was observed in the development of edema formation during the acute phase of the disease in these mice compared with wt mice. These data indicate that even in the absence of the CD28 T cell costimulatory molecule an immune response against Bb is taking place, implying that other molecules provide proper costimulation. This result is consistent with a previous report that CD80/CD86 blockade in B6 mice did not interfere with acute arthritis (28). IL-10 is an anti-inflammatory cytokine, induced by Bb lipopeptides, that negatively regulates the immune response against Bb by inhibiting the production of proinflammatory cytokines by macrophages (28). IL-10<sup>−/−</sup> mice on an arthritis-resistant B6 background showed increased acute arthritis upon Bb infection (8) (29).

In our system, the acute inflammatory response was not affected, because no differences in the ankle width were observed between the CD28<sup>−/−</sup> and the control group during the early phase of the disease. Likewise, when CD28<sup>−/−</sup> mice were infected with Plasmodium chabaudi parasites, the acute response was similar to that of wt B6 mice. However, the P. chabaudi infected CD28<sup>−/−</sup> mice developed chronic parasitemia, compared with the control mice which cleared the parasites (30).

The prevalence of the observed recurring monoarticular arthritic episodes in the Bb-infected CD28<sup>−/−</sup> mice is even more significant, because these mutants are on the B6 background, which is relatively resistant to Bb-induced arthritis. The intermittent inflammatory reactions and remission phases might reflect an immune response against different Bb variants. Bb has the ability to modulate the expression of its highly immunogenic outer surface proteins (31). It is possible that in an environment where immunoregulatory mechanisms are compromised, expression of Bb variants might flare up the host immune response.

Upon the investigation of the humoral response to Bb Ags, CD28<sup>−/−</sup> mice showed decreased titers of anti-Bb Abs compared with the control mice. This outcome was anticipated in light of the observation that the basal IgG levels are reduced in the CD28<sup>−/−</sup> mice (15). However, despite the overall lower humoral response to Bb lysate, the mutant mice, but not the control mice, mounted a significant Ab response against OspA. Furthermore, the anti-OspA Abs in the CD28<sup>−/−</sup> mice developed only after the establishment of chronic Lyme arthritis. This is particularly interesting, because OspA, mainly expressed in the midgut of Bb-infected ticks, gets down-regulated upon feeding of the infected tick on a mammalian host (32). Thus, it is not surprising that anti-OspA Abs are barely detectable in mice during acute arthritis, nor in patients during early Lyme arthritis (24, 33, 34). Nevertheless, patients with chronic Lyme arthritis are seropositive for OspA Abs, and this humoral response parallels the severity and duration of arthritis (24, 25). Collectively these studies suggest that OspA expression gets up-regulated in the mammalian host during a chronic inflammatory reaction, a hypothesis that we were able to confirm in vivo (35). Our results fit with this model and provide additional evidence for the notion that perpetual inflammation is a prerequisite for the development of an anti-OspA humoral immune response.

The majority of chronic Lyme arthritis patients clear arthritis upon antibiotic treatment. In contrast, 10% of these patients continue to show inflammation of their joints, a condition termed treatment-resistant Lyme arthritis (TRLA) (36). When Bb-infected CD28<sup>−/−</sup> mice were treated with ceftriaxone, an antibiotic that has been reported to effectively eradicate Bb (37, 38), all animals cleared arthritis, with no signs of recurring joint inflammation for at least 2½ months post antibiotic therapy. This finding, however, was not surprising, since it is well established that the risk of developing TRLA is highest in individuals with rheumatoid arthritis-associated HLA.DR alleles, mainly of HLA.DR4 haplotype (39). In these TRLA patients the magnitude of the anti-OspA immune response correlates with the severity of Lyme arthritis, even in the absence of active Bb infection (4, 25). This observation led to the hypothesis that an autoimmune response is induced in these individuals. Indeed, we have recently shown that the presence of a HLA.DR4 transgene into CD28<sup>−/−</sup> mice allows the development of TRLA (manuscript submitted for publication).

In conclusion, we have generated a mouse model for chronic Lyme arthritis. This murine system addresses the role of immunoregulation in the development of chronic Lyme arthritis and provides a tool for the dissection of the immunological manifestation that perpetuates chronic joint inflammation due to Bb infection.

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Disclosures

The authors have no financial conflict of interest.

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