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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. Huber‡*‡

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), 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...
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−/− mouse (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 in 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 tibular area of both hind limbs (1 cm above the knee) with a total dose of 2 × 10^7 Bb/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 Decalcifier 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 Proteinase 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-isomyl alcohol 25:24:1 (Invitrogen) and last by an equal volume of chloroform (Fisher Scientific). 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 (Minor Groove binder) probes for Bb RecA (GenBank accession no. U23457) were designed and used for Bb detection (F-5′-TGCGTAGCCTTCCCTATTTGATGT-3′, R-5′-GAATAGCCTTACGTGAACTTCAAG-3′, FAM-5′-TGCGTAGCCTTCCCTATTTGATGTA-3′, R-5′-GACTTTAGGCTGCTGACGAC-3′, R-3′-AGCTTTGATGTGCAAAAGCTTTAGGCTGCTGACGAC-3′). The amount of template DNA was first extracted by 250 μl of buffer-saturated phenol (Invitrogen), then by an equal volume of phenol-chloroform-isomyl alcohol 25:24:1 (Invitrogen) and last by an equal volume of chloroform (Fisher Scientific). 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 (Minor Groove binder) probes for Bb RecA (GenBank accession no. U23457) were designed and used for Bb detection (F-5′-TGCGTAGCCTTCCCTATTTGATGT-3′, R-5′-GAATAGCCTTACGTGAACTTCAAG-3′, FAM-5′-TGCGTAGCCTTCCCTATTTGATGTA-3′, R-5′-GACTTTAGGCTGCTGACGAC-3′, R-3′-AGCTTTGATGTGCAAAAGCTTTAGGCTGCTGACGAC-3′). The amount of template DNA was first extracted by 250 μl of buffer-saturated phenol (Invitrogen), then by an equal volume of phenol-chloroform-isomyl alcohol 25:24:1 (Invitrogen) and last by an equal volume of chloroform (Fisher Scientific). 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 (Minor Groove binder) probes for Bb RecA (GenBank accession no. U23457) were designed and used for Bb detection (F-5′-TGCGTAGCCTTCCCTATTTGATGT-3′, R-5′-GAATAGCCTTACGTGAACTTCAAG-3′, FAM-5′-TGCGTAGCCTTCCCTATTTGATGTA-3′, R-5′-GACTTTAGGCTGCTGACGAC-3′, R-3′-AGCTTTGATGTGCAAAAGCTTTAGGCTGCTGACGAC-3′). The amount of template DNA was first extracted by 250 μl of buffer-saturated phenol (Invitrogen), then by an equal volume of phenol-chloroform-isomyl alcohol 25:24:1 (Invitrogen) and last by an equal volume of chloroform (Fisher Scientific). 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 (Minor Groove binder) probes for Bb RecA (GenBank accession no. U23457) were designed and used for Bb detection (F-5′-TGCGTAGCCTTCCCTATTTGATGT-3′, R-5′-GAATAGCCTTACGTGAACTTCAAG-3′, FAM-5′-TGCGTAGCCTTCCCTATTTGATGTA-3′, R-5′-GACTTTAGGCTGCTGACGAC-3′, R-3′-AGCTTTGATGTGCAAAAGCTTTAGGCTGCTGACGAC-3′)

Anti-Bb and anti-OspA ELISA

Flat bottom Immulon 2HB plates (Fisher Scientific) were coated overnight with 10 μg/ml Bb lysozyme or 5 μg/ml recombinant OspA (0.1 M Na.HPO4 (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 × 10^7 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.
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 the Student’s t test (if two groups are compared), and ANOVA (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.

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 (\pm SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28(^{-/-})</td>
<td>25/28*</td>
<td>3.2 (\pm 0.14)</td>
</tr>
<tr>
<td>B6</td>
<td>7/29</td>
<td>2.9 (\pm 0.21)</td>
</tr>
</tbody>
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\(\ast CD28^{-/-} (n = 28) and wt B6 mice (n = 29) were infected with 2 \times 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.

\(n = 7(two-tailed Fisher exact probability test).\)

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 \times 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.
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 chronic phase of Lyme arthritis and tested by real-time qPCR for the level of the RecA gene, similarly to wt mice and wt controls. DNA was isolated from the ear punches of the infected mice as well. As shown in Fig. 5A, by day 200 post infection the CD28−/− mice developed lower anti-Bb IgG titers than 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 Abs.
IgM and IgG3 isotypes. We also observed that the CD28 decrease in IgG1, IgG2a, and IgG2b isotypes and an increase in (Fig. 6A), although recurring arthritic episodes presented before this date (Fig. 6B). 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...

In addition, as seen in Fig. 6, A and B, the CD28−/− mice mounted anti-OspA Abs only after the establishment of chronic arthritis, implying that perpetuated inflammation is a prerequisite for the development of an anti-OspA humoral immune response. Indeed, anti-OspA Ab titers were detectable only 200 days post Bb infection in the majority of CD28−/− mice (Fig. 6A), even though recurring arthritic episodes presented before this date (Fig. 6B). 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.

**FIGURE 6.** The development of anti-OspA Abs in the CD28−/− mice requires the establishment of chronic Lyme arthritis. The anti-OspA Ab titers (A) as well as the ankle width (B) were monitored over the course of 200 days, in Bb-infected CD28−/− mice (n = 18). The horizontal line in B represents the ankle width of mock-infected CD28−/− mice. Absolute numbers represent mice with anti-OspA Ab (total mice (A) and arthritic mice (B)). Even though CD28−/− mice develop arthritis early on, anti-OspA Abs are detected after a prolonged period of chronic Lyme arthritis. Data include the pooled results of four independent experiments.

**FIGURE 7.** Chronic Lyme arthritis in CD28−/− mice resolves upon antibiotic treatment CD28−/− (n = 4) and wt (n = 4) were infected with 2 × 10^4 Bb/mouse. Mock-infected mice (n = 2) are represented by the open squares. Arthritis was assessed periodically by measuring the ankles using a caliper. On day 70 post infection, both groups were treated with ceftriaxone (50 mg/kg/dose, for 5 days), and arthritis was monitored for 2.5 mo post antibiotic treatment. Graph depicts the average ankle width (mm). Error bars, SEM. Asterisks indicates a significant difference in ankle swelling between the CD28−/− and the B6 mice (p < 0.001; Student’s two-tailed, unequal variance t test). Statistical significance has been also confirmed based on the AUC for each group (p = 0.02).
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−/− mice after infection with Bb, because these mutant mice have a substantial reduction in the number of Treg 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 taking place, implying that other molecules provide proper co-stimulation. IL-10 proteins, that negatively regulates the immune response against the CD28

molecules are regulated in the development of chronic Lyme arthritis and provide 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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