Borrelia Burgdorferi Basic Membrane Protein A Initiates Arthritis in Tree Shrews

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Abstract

Borrelia burgdorferi Basic membrane protein A (BmpA) plays a crucial role in mediating immune responses to Lyme arthritis. The aim of this study was to describe manifestations and the main evaluation method on recombinant BmpA (rBmpA) - induced arthritis in tree shrews. We used rBmpA to stimulate knee joint in vivo in tree shrew animals. Arthritis was evaluated by Arthritis index, pathology, expression of proinflammation cytokines. Results showed that the joints of tree shrews appeared swelling, both the scores of arthritis and the number of swelling joints were enhanced The levels of TNF-α and CXCR4 increased in Peripheral blood. Day 7-15 after BmpA injection, the inflammation reached its peak. Histopathology examination showed that the synovium was hyperplasia, arranged irregularly. Our data indicated that the rBmpA-induced tree shrew arthritis may be used as a candidate model for Lyme arthritis pathogenesis and treatment.

Keywords: Lyme arthritis; *Borrelia burgdorferi*; rBmpA; Tree threw.

Introduction

Lyme Disease (LD), a serious inflammatory syndrome caused by the *spirochete Borrelia burgdorferi* (*B. burgdorferi*, Bb), is characterized by tissue inflammation of the skin, muscles, joints, and central nervous system (CNS). Lyme disease occurs throughout North America, Europe (particularly central Europe and Scandinavia, Russia), and Asia [1]. Since Lyme disease was recognized in 1976 [2], 300,000 cases have been reported annually in the United States [3,4].

Lyme arthritis is inflammatory arthritis that is likely mediated by the immune response to the spirochete in the joint and can be seen in the early or late stage of Lyme disease [5]. Lyme arthritis has been characterized as a mono- or oligoarthritis, typically presenting as a monoarthritis of the knee [6,7]. Patients with Lyme disease could suffer from varying degrees of pain and swelling of the knee as well as skin involvement with redness and oedema around the ankle of the same leg [8,9].

BmpA is a major membrane protein that is closely associated with Lyme arthritis [10]. BmpA, a specific, highly conserved, and immunogenic lipoprotein, is encoded by the *bmp* family genes of paralogous chromosomal gene family 36 [11,12]. BmpA operons that are induced in murine and human joints possess inflammatory properties. BmpA-deficient *B. burgdorferi* persists poorly in joints and fails to induce arthritis compared to the wild type *B. burgdorferi* [13]. An isogenic BmpA mutant induced significantly lower levels of pro-inflammatory cytokines TNF-alpha and IL-1beta in cultured human synovial cells. BmpA can induce cytokine responses in synovial cells via activation of the NF-κB and p38 MAP kinase pathways [14].

In the past 45 years, a wide variety of animals have been used to study the pathogenesis of Lyme arthritis and determine therapeutic targets. These animals include rodents [15] that are most used (C3H/HeN mice, C57BL/6J mice, Gamma interferon gene-deficient mice, C3H/HeN-SCID mice, BALB/C mice), hamsters and nonhuman primates [13,16-22]. Although the current infection models of Lyme arthritis are useful in understanding the basic immunological events that lead to the development of *Borrelia burgdorferi*-induced inflammation, these findings need confirmation in humans [23]. High costs limit the routine use of large non-human primates. The Northern Tree shrew (*Tupaia belangeri*) is a small squirrel-like mammal indigenous to southwest Asia. Compared with rodents, the Tree shrew genome shows higher similarity to humans, and many of their biological characteristics have close homology with humans [24]. Importantly, from the perspective of infectious diseases, tree shrews are susceptible to infection with human pathogens and manifest clinical signs akin to human infections [25,26]. Therefore, using *Borrelia burgdorferi* bmpA to induce the occurrence of arthritis in tree threw could be a better choice.

Materials and methods

Ethics statement

Tree shrews were obtained from the Kunming Medical University, Yunnan, China* (https://www.kmmc.cn/list1370.aspx). The animals were bred and maintained at the Animal Laboratory Center of Kunming Medical University in strict accordance with the rules and regulations of China animal welfare law. All animal experiments were approved by the Experimental Animal Committee of Yunnan (Permit Numbers: LA2021744, JXJY20200018).

rBmpA preparation

E. coli expression system was used to produce recombinant BmpA (rBmpA) using the bacterial expression plasmid vector pGEX- 6P-1 (GE, USA) (Figure 1), and the following BmpA primers was used (5'-3'containing EcoRI and XhoI restriction sites): forward, ACG AAT TCA TGA ATA AAA TAT TGT TGT TGA, reverse, AGC TCG TAA ATA AAT TCT TTA AGA AA. Expression, purification, and enzymatic cleavage of the glutathione transferase fusion protein were carried out as described before [10].

Animal preparation

Thirty-two adult female Tree shrews (4-6 months old, 150 g mean weight). Animals were routinely fed in a isolated cages in an experimental animal room under the following conditions: 25±2°C temperature, 50% relative humidity, natural ventilation 8~10 h/d, noise <60 dB, and 12h/12h light/dark cycle.

Induction of arthritis

The purified rBmpA protein was diluted with 0.01M PBS and injected into the left knee joints of tree shrews following different concentrations: Low dose groups (0.04 mg/ml, 50 μL), high dose groups (0.08 mg / ml, 50 μL), PBS groups (0.01M, 50 μL). All tree shrews were then examined daily for joint swelling and assigned a clinical arthritis score (Figure 1).

Arthritis scoring

The arthritis scores were scored twice a week by two people who were not sure about the experimental group. Arthritis index score criteria (0-4) were as follows [27]: 0, no swelling or inflammation; 1, slight swelling and/or redness of paw; 2, mod-erate swelling in joints; 3, pronounced swelling with more than two joints; and 4, severe swelling with joint rigidity. The arthritis score was the sum of the affected joints in each tree shrew's hind limbs.

Tissue collection and histopathologic analysis

The tree shrews in each group (n=8) were euthanized by CO_2 asphyxia on the 28th day after injection. The hind limbs of each animal were immediately fixed in 10% formalin, then decalcified, implanted with paraffin, sectioned and stained with haematoxylin-eosin. The slides were observed under high power microscope (×40) taken photos.

Tree shrew blood cytokines detection

The heart blood was collected from Tree shrews on the 7th, 14th, and 21st days after the injection. Total RNA was extracted from whole blood using RNAiso Plus reagent (TaKaRa). A Prime Script RT reagent Kit with Gdna Eraser (TaKaRa) was used to synthesize cDNA, according to the manufacturer's protocol. Real-time PCR was conducted using SYBR Premix Ex Taq (TaKaRa), according to the manufacturer's protocol. Relative gene expression levels were calculated using the comparative threshold cycle method for quantitative PCR, with data normalized to the housekeeping gene GAPDH. Sequences of the specific primers used to amplify GAPDH, TNF-α, IL-2, IL-6, IL-10, IL-17, CXCR4 are listed in Table 1.

Table 1: Sequences of specific primers used for quantitative RT-PCR.

Gene	Forward primer (5´→3´)	Reverse primer (5´→3´)
GAPDH	GGTGCCGAGTATGTTGTGGA	TCTCATGGTTCACGCCCATC
TNF-α	CGCTACCTGGACTTTGCTGA	CCCTGAGCCCCTAATTCCCT
IL-2	CGTCACAAACAGTGCACCTAC	CCTGGAGAGCATCTTTGGGTT
IL-6	CGTCACAAACAGTGCACCTAC	CCTGGAGAGCATCTTTGGGTT
IL-10	AAACCAAACCACAAGGGCAC	AGGTTGGCTGGGAAGTAGGT
IL-17A	CTGTGTGGATGCTGAAGGGA	CAGCCCACAGTCACCAGTAT
CXCR4	GACTTGTGGGTGGTAGTGTTCC	GACTGTGGTCTTCAGGGCTTTG

Statistical analysis

All data are shown as means ± Standard Deviation (SD). Statistical analysis were performed using GraphPad Prism 9.2.0 software (GraphPad Software Inc, San Diego, CA). Two-way Analysis Of Variance (ANOVA) was used for comparison of multiple groups, followed by Tukey's multiple comparison test for post hoc test. $P < 0.05$ was set as the level of statistical significance.
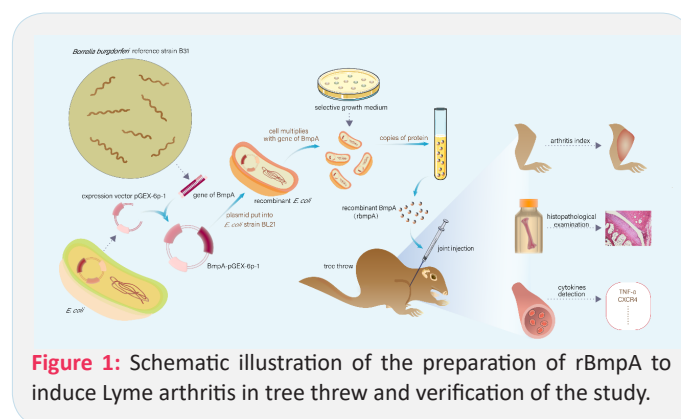

Figure 1: Schematic illustration of the preparation of rBmpA to induce Lyme arthritis in tree threw and verification of the study.

Results

Animal observation

The tree shrews in the healthy control group (Figure 2A) and the PBS control group had normal feeding, flexible activities, no "wet tail" caused by diarrhea, no bleeding at the injection site, no rash, and slight redness and swelling at the local injection site 24h after the injection lasted only 2 to 3 days. No other abnormality post-injection was seen by the naked eye (Figure 2A,B). In experimental group, food intake, activity, and body response of the tree shrews were generally reduced on day 7 after rBmpA injection, which manifested as often animals hiding in cages and not easy to be driven.

The degree of joint swelling in rBmpA-injected tree shrew group

The knee joint swelling and congestion in the low-dose rBmpA group were all obvious. The knee joint swelling in the high-dose rBmpA group was more clear, but the congestion was not obvious (Figure 2C,2D). Both rBmpA groups reached the peak of lesions in 7-15 days, and then the symptoms gradually alleviated after 3 week (Figure 2E). rBmpA groups have small ulcers on their knee joints after rBmpA injection, and the scabs can be repaired in about 2-3 days. The stool under the cage was observed to be green and loose. After anatomy, it was found that the intestines were dark. It may be caused by enteritis, diarrhea, and dehydration. Our evidence showed that there were no obvious lesions on the front paws, tails, and other surfaces of the tree shrews in each group. One week after the injection, the incidence of arthritis in the experimental group reached 80%, and the arthritis score was 2-6. The average arthritis index reached a peak two weeks after the injection (Figure 2F). The clinical symptoms in the high-dose group are easier to observe. The tree shrews in the health control group (Figure 2A) and PBS group (Figure 2B) showed no swelling and congestion of hind limb.
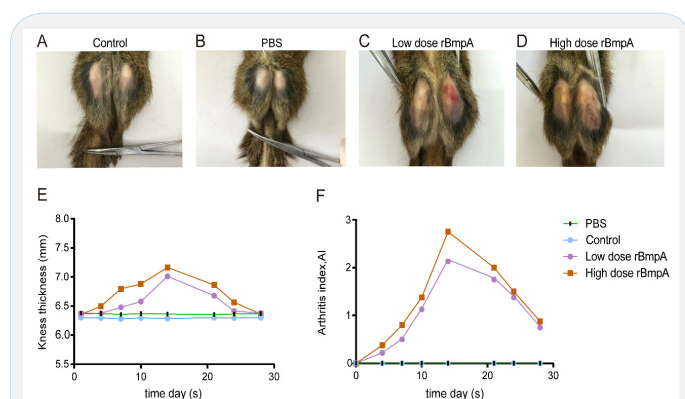

Figure 2: The degree of joint swelling in the tree threws rBmpA group was obvious. **(A-D)** The images of each group were collected on the 14th day after the injection was stopped. Compared with the healthy control **(A)**, the PBS group **(B)** showed no signs of joint inflammation and other infections. In representative pictures of the left hind limb injection of rBmpA 2ug **(C)** and 4ug **(D)** directly injected on the 14th day are shown. **(E)** Time course of joint swelling of the left hind limb with direct injection (joint thickness in millimeters). **(F)** Dynamic changes of the average arthritis index of each group in tree shrew.

Injection of rBmpA triggered synovial cartilage degeneration

All tree shrews were euthanized on day 28 postinjection. Both hind limbs of each euthanized animal were immersion-fixed, decalcified, and stained with hematoxylin-eosin for his-

topathological evaluation. Tree shrews in the rBmpA groups (Figure 3C,3D) showed early manifestations of cartilage degeneration, with normal joint spaces, irregular articular surfaces, depressions in the synovial cartilage of the joints, and irregular cell arrangement in the depressions. In addition, the irregular depressions or line-like grooves on the surface of articular cartilage showed chondrocyte proliferation and cartilage microfibrosis. Such articular cartilage degeneration were absent in control group injected with PBS (Figure 3B). Our data indicate that local exposure of the joints to rBmpA is sufficient to trigger the early pathological changes of arthritis.
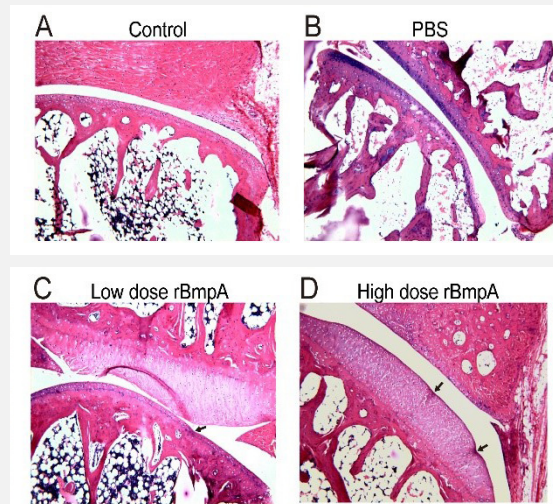

Figure 3: Injection of rBmpA triggers synovial cartilage degeneration in tree shrews. Representative optical micrograph of hematoxylin-eosin-stained sections of tree shrew joint collected on the 28th day after administration. **(A)** The uninjected healthy control tree shrews had no histopathological features. **(B)** PBS groups lacked histopathological features when examined at the same time point. **(C)** Low dose rBmpA groups showed a single local joint synovial cartilage depression, and the cells in the depression site are not aligned rule (arrow). **(D)** High dose rBmpA groups showed joint multiple synovial cartilage degeneration, joint synovial cartilage depression, irregular cell arrangement in the depression, Cartilage cell hyperplasia, and cartilage microfibrosis (arrow).
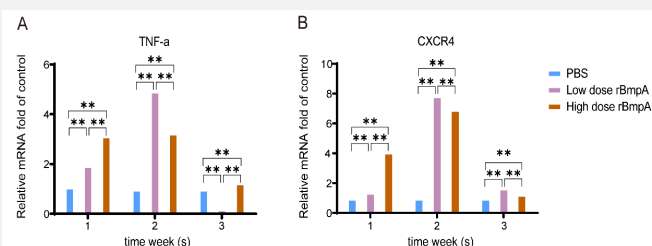

Figure 4: Significant alteration of TNF and CXCR4 mRNA expression from peripheral blood of tree threws after rBmpA induction at 1, 2, and 3 week. Statistically significant differences between groups (P<0.01). **P<0.01, *P<0.05. Analyzed with two-way ANOVA and data were expressed as the means ± SD. Abbreviations: TNF, tumor necrosis factor α; CXCR4, chemokine CXC receptor 4; rBmpA, recombinant *Borrelia burgdorferi* basic membrane protein A.

The expression of TNF-α and CXCR4 in tree shrews

To further study pathogenesis of rBmpA-induced arthritis, the expression levels of IL-2, IL-6, IL-10, IL-17A, TNF-α, and CXCR4 mRNA in peripheral blood of tree shrews were determined by RT-PCR following rBmpA injection. Compared with the untreated control group, TNF-α and CXCR4 mRNA expres-

sion levels in tree shrew peripheral blood increased significantly after rBmpA stimulation for 1,2, and 3w, reaching a peak at 2 weeks. However, there were no significant differences in IL-2, IL-6, IL-10, and IL-17A mRNA expression levels in experimental group relative to the control group at any time point.

Discussion

The pathogenesis of Lyme arthritis is still under exploration. Our study confirmed that using rBmpA to inject into knee joint of tree shrews can induce Lyme arthritis.

Tree shrew as an animal model broadens the limitations of Lyme arthritis research. Since tree shrews are closer to humans, we used tree shrews to simulate the characteristics of human Lyme arthritis. So far, *Borrelia burgdorferi* has been used to in-fect variety of animal models, including rodents [28-30], rab-bits [31,32], dogs [33,34], monkeys [35] horses [36], cattle [37] and people, etc [38]. However, the animal models for Lyme ar-thritis are mainly mice. These mice provide important insights and valuable knowledge for understanding the pathogenesis of Lyme arthritis. Although mice are widely used because of their small size and clear genetic background, there are significant genetic differences between rodents and humans. Be cautious when translating these findings directly into the human system [39]. Mice sets limitations for the study of human Lyme arthri-tis. Non-human primates are genetically close to humans, and they can simulate human physiology and pathology relatively accurately. However, their high cost and low productivity also severely limit their use. Human body experiments, due to issues related to the maintenance of human ethics, and the difficulty of recruiting volunteers, hinder the possibility of its implemen-tation. Compared with other experimental animals, tree shrew genome sequencing showed that its neural, immune, and meta-bolic systems are very close to humans, and it is a small mam-mal closely related to primates. It has been proposed as an al-ternative laboratory animal to primates in biomedical research [40].

Our research is to use rBmpA instead of live *Borrelia burg-dorferi* as an inducer for its close relationship with Lyme disease (Figure 1). At present, the establishment of animal models of Lyme arthritis is mainly carried out by subcutaneous injection of live *Borrelia burgdorferi* [41]. This induction method is intended to simulate the natural transmission method of tick bites to infect animals, but some animal studies also used needle in-oculation instead of tick transmission to infect animals, com-pared to the natural method of tick bites. It have found that, For spirochetes that enter animals in this way, the eradication of *Borrelia burgdorferi* with antibiotics may not be complete. *Bor-relia burgdorferi* DNA can be detected in animals tissues up to 9 months after antibiotic treatment [34,35,42-44]. In the past, it was believed that if this kind of tissue residual spirochetes exists in humans, it is the cause of post-Lyme disease syndrome (persistent musculoskeletal pain, neurocognitive symptoms, or fatigue feelings after antibiotic treatment, etc.) [45-47]. How-ever, the current view believes that there is a serious method-ological problem that does not consider the pharmacokinetics of antibiotics when choosing the dosage regimen used Kinetic-pharmacodynamic properties [48], the pathogenesis of later features of LD may no longer be dependent on infection [49]. The study found that BmpA is one of the exposed and highly antigenic proteins on the surface of *Borrelia burgdorferi*. BmpA can induce cytokine response in synovial cells. NF-κB and p38 MAP kinase pathway activate the pro-inflammatory response of human synovial cells [14]. In mice, it may trigger a complex

cascade of host inflammatory responses that cause arthritis. It was found that the expression levels of *Borrelia burgdorferi* bmpA and bmpB were significantly higher than that of the skin, suggesting that bmpA may also be applicable to Lyme arthri-tis model [13]. Our previous studies have also shown that the basic membrane protein A of *Borrelia burgdorferi* triggers a storm of pro-inflammatory chemokines in THP1-derived mac-rophages through receptors TLR1 and TLR2, which may trigger innate and adaptation in infected arthritis [10]. Therefore, on the one hand, in order to avoid the controversy of Lyme disease syndrome caused by residual spirochetes, we can use rBmpA to replicate Lyme arthritis model [13,14,50].

rBmpA induces pathological changes in the synovium of tree shrew joints (Figure 3). The pain caused by human Lyme arthritis can be seen in the early or late stage, the early stage is mainly proliferative synovitis [51], and some patients in the late stage can develop chronic and severely destructive Lyme arthritis [8], and its pathological characteristics include synovial hyperplasia, infiltration of monocytes into the synovium, forma-tion of inflammatory exudates on the synovial cell wall (vascu-lar loop formation), cartilage destruction, and bones and joints [52,53]. C3H mice, the most commonly used animal models for Lyme arthritis [23], whose severity of this arthritis usually peaks around 3 weeks after the Intradermal inoculation of susceptible strains, after which the pathological changes quickly subside. In addition, joint edema changes are usually accompanied by his-tological changes. In the infection model of Lyme arthritis mice, this swelling can be observed about 2 weeks after infection and gradually decreases. In our study, the degree of joint swelling and arthritis index of tree shrews reached their peak at 2 weeks after being infected with rBmpA, and then gradually decreased (Figure 2). The pathology of the tree shrew's joints is that the early manifestations of cartilage degeneration appear after 4 weeks (Figures 3C and 3D).the articular surface is irregular, the joint synovial cartilage is recessed, the cell arrangement of the recessed area is irregular. Because it is a preliminary explora-tion, the observation time is also being explored. If continuous long-term observation is continued, further research can be done on whether rBmpA can cause persistent Lyme disease ar-thritis.

In this study, we found that increased TNF-α (Figure 4A) and CXCR4 (Figure 4B) in the blood of the tree threws in response to rBmpA of *Borrelia Spirochetes*. An previous study reported that rBmpA initiates proinflammatory chemokine storm in THP 1-derived macrophages in humans via the receptors TLR1 and TLR2 [10]. TLRs are a conservative molecular model in which the receptors of the host cell can recognize microbial compo-nents such as lipoproteins, lipopolysaccharides, flagellin, and nucleic acids [54]. The rBmpA used in our study is one of the lipoprotein molecules on the surface of the *Borrelia burgdor-feri* membrane, which may be recognized by TLRs to initiate the innate immune response. Studies have shown an increase in transcript levels of genes encoding CC and CXC chemokines, proinflammatory cytokines, and TNF superfamily members in the joint tissues infected with live *Borrelia burgdorferi*-infected mice [55-57]. Under the stimulation of *Borrelia burgdorferi*, neutrophils promote the development of arthritis by releasing pro-inflammatory cytokines, including TNF-A, IL-1, IL-8, IL-15, and so on [58,59]. Chemokines can regulate leukocyte traffick-ing during host defense immune response [60]. CXCR4 is one of the receptors of the chemokine subfamily CXC, a typical G Protein-Coupled Receptor (GPCR) [64,65], which can activate G-protein-mediated downstream signaling pathways [66], whose

ligand is CXCL12, an important member of the CXC chemokines [16]. The CXCL12-CXCR4 axis is well established to play multiple roles in cellular migration, survival, and proliferation, as well as in other functions [61-63]. CXCL12 and CXCR4 were highly expressed in the serum and joint synovial fluid of patients with Rheumatoid Arthritis (RA) [64]. CXCR4 levels in synovium have been correlated with joint destruction in RA patients [65]. The subset expressing CXCR4 in the peripheral blood positively correlated with TNF-α in RA patients [66]. TNF-α has involved the occurrence of inflammation and the pathogenesis in RA [67,68]. TNF-a and CXCR4 reached their peaks 2 weeks after rBmpA inoculation (Figure 4), which was consistent with the process of joint swelling (Figure 2). Our experiment suggests that CXCR4 and TNF-α may be involved in the inflammation of Lyme arthritis.

Moreover, studies have shown that CXCR4 was both relatively highly expressed in the spleen and thymus of tree threws, which implying that CXCR4 may be involved in some aspect of immunological regulation among tree shrews. The study showed that CXCL12 and CXCR4 in tree threws are structurally similar to their homologous human proteins. The similarities between tree shrew and humans CXCR4 were as high as 97%, and those of mice and rats were 91% and 92%, respectively [69]. This shows that the expression of CXCR4 in tree shrews is closer to humans than in mice and rats. Tree shrews might be more suitable for explaining human clinical phenomena. Our experiment implicated that tree shrews CXCR4 level increase during joint swelling (Figure 2) may play similar roles as those in humans Lyme arthritis. Tree shrew may be a viable animal model for studying human Lyme arthritis.

In the next step, we plan to extend the observation time of tree shrew model, increase the induction mode of rBmpA and use more experimental verification methods which is beneficial to the research of Lyme arthritis.

Conclusion

Our study identifies rBmpA as a likely contributor to inflammatory responses in vivo in tree threw arthritis. Persistence of this antigen in the joint may lead to synovitis. Using new method of to simulate Lyme arthritis of tree shrews with rBmpA immunogen helps to expand Lyme arthritis model spectrum. The expression of tumor necrosis factor-α and CXCR4 gene in whole blood of tree shrews increased significantly and could correlate to Lyme arthritis pathogenesis. Manifestations in tree shrew arthritis suggests a potential role of rBmpA in the pathogenesis of Lyme arthritis.

Declarations

Author contributions: Fukai Bao, Aihua Liu, and Bingxue Li conceived and designed the experiments. Yuxin Fan, Shiyuan Wen developed the methodology. Bingxue Li, Hanxin Wu, Weijie Ma, Lei Zhong, Bingxue Li, Li Peng, Li Gao, Xinya Wu, and Weijiang Ma performed all experiments. Weijiang Ma, Liangyu Zhu, Jieqin Song, Suyi Luo analyzed and discussed the data. Bingxue Li wrote the manuscript. Fukai Bao, Aihua Liu, and Bingxue Li edited and revised the manuscript. All authors read and approved the final manuscript.

References

1. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet. 2012; 379: 461-473.

2. Steere AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, et al. Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum. 1977; 20: 7-17.

3. Mead PS. Epidemiology of Lyme disease. Infect Dis Clin North Am. 2015; 29: 187-210.

4. Nelson CA, Saha S, Kugeler KJ, Delorey MJ, Shankar MB, et al. Incidence of Clinician-Diagnosed Lyme Disease, United States, 2005-2010. Emerging Infect Dis. 2015; 21: 1625-1631.

5. Schoen RT. Lyme disease: Diagnosis and treatment. Curr Opin Rheumatol. 2020; 32: 247-254.

6. Steere AC, Strle F, Wormser GP, Hu LT, Branda JA, et al. Lyme borreliosis. Nat Rev Dis Primers. 2016; 2: 16090.

7. Brouwer MAE, van de Schoor FR, Vrijmoeth HD, Netea MG, Joosten LAB. A joint effort: The interplay between the innate and the adaptive immune system in Lyme arthritis. Immunol Rev. 2020; 294: 63-79.

8. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. Ann Intern Med. 1987; 107: 725-731.

9. Herzer P. Diagnosis and therapy of Lyme arthritis. Schweiz Rundsch Med Prax. 1993; 82: 393-398.

10. Zhao H, Dai X, Han X, Liu A, Bao F, et al. Borrelia burgdorferi basic membrane protein A initiates proinflammatory chemokine storm in THP 1-derived macrophages via the receptors TLR1 and TLR2. Biomed Pharmacother. 2019; 115: 108874.

11. Gorbacheva VY, Godfrey HP, Cabello FC. Analysis of the bmp gene family in Borrelia burgdorferi sensu lato. J Bacteriol. 2000; 182: 2037-2042.

12. Simpson WJ, Cieplak W, Schrumpf ME, Barbour AG, Schwan TG. Nucleotide sequence and analysis of the gene in Borrelia burgdorferi encoding the immunogenic P39 antigen. FEMS Microbiol Lett. 1994; 119: 381-387.

13. Pal U, Wang P, Bao F, Yang X, Samanta S, et al. Borrelia burgdorferi basic membrane proteins A and B participate in the genesis of Lyme arthritis. J Exp Med. 2008; 205: 133-141.

14. Yang X, Izadi H, Coleman AS, Wang P, Ma Y, et al. Borrelia burgdorferi lipoprotein BmpA activates pro-inflammatory responses in human synovial cells through a protein moiety. Microbes Infect. 2008; 10: 1300-1308.

15. Tuttle C. Post-Lyme Arthritis May Be More Than Lyme: Comment on the Article by Arvikar et al. Arthritis Rheumatol. 2017; 69: 684.

16. Kang I, Barthold SW, Persing DH, Bockenstedt LK. T-helper-cell cytokines in the early evolution of murine Lyme arthritis. Infect Immun. 1997; 65: 3107-3111.

17. Kuo J, Nardelli DT, Warner TF, Callister SM, Schell RF. Interleukin-35 enhances Lyme arthritis in Borrelia-vaccinated and -infected mice. Clin Vaccine Immunol. 2011; 18: 1125-1132.

18. Anguita J, Samanta S, Barthold SW, Fikrig E. Ablation of interleu-

kin-12 exacerbates Lyme arthritis in SCID mice. Infect Immun. 1997; 65: 4334-4336.

19. Schaible UE, Kramer MD, Museteanu C, Zimmer G, Mossmann H, et al. The severe combined immunodeficiency (scid) mouse. A laboratory model for the analysis of Lyme arthritis and carditis. J Exp Med. 1989; 170: 1427-1432.

20. Schmitz JL, Schell RF, Hejka A, England DM, Konick L. Induction of lyme arthritis in LSH hamsters. Infect Immun. 1988; 56: 2336-2342.

21. Hejka A, Schmitz JL, England DM, Callister SM, Schell RF. Histopathology of Lyme arthritis in LSH hamsters. Am J Pathol. 1989; 134: 1113-1123.

22. Barthold SW, Moody KD, Terwilliger GA, Jacoby RO, Steere AC. An animal model for Lyme arthritis. Ann N Y Acad Sci. 1988; 539: 264-273.

23. Nardelli DT, Callister SM, Schell RF. Lyme arthritis: Current concepts and a change in paradigm. Clin Vaccine Immunol. 2008; 15: 21-34.

24. Fan Y, Huang ZY, Cao CC, Chen CS, Chen YX, et al. Genome of the Chinese tree shrew. Nat Commun. 2013; 4: 1426.

25. Cao J, Yang EB, Su JJ, Li Y, Chow P. The tree shrews: Adjuncts and alternatives to primates as models for biomedical research. J Med Primatol. 2003; 32: 123-130.

26. Tsukiyama-Kohara K, Kohara M. Tupaia belangeri as an experimental animal model for viral infection. Exp Anim. 2014; 63: 367-374.

27. Li Y, Zheng JY, Liu JQ, Yang J, Liu Y, et al. Succinate/NLRP3 Inflammasome Induces Synovial Fibroblast Activation: Therapeutical Effects of Clematichinenoside AR on Arthritis. Front Immunol. 2016; 7: 532.

28. Yrjänäinen H, Hytönen J, Hartiala P, Oksi J, Viljanen MK. Persistence of borrelial DNA in the joints of Borrelia burgdorferi-infected mice after ceftriaxone treatment. APMIS. 2010; 118: 665-673.

29. Yrjänäinen H, Hytönen J, Song XY, Oksi J, Hartiala K, et al. Antitumor necrosis factor-alpha treatment activates Borrelia burgdorferi spirochetes 4 weeks after ceftriaxone treatment in C3H/He mice. J Infect Dis. 2007; 195: 1489-1496.

30. Feng J, Li T, Yee R, Yuan Y, Bai C, et al. Stationary phase persister/biofilm microcolony of Borrelia burgdorferi causes more severe disease in a mouse model of Lyme arthritis: implications for understanding persistence, Post-treatment Lyme Disease Syndrome (PTLDS), and treatment failure. Discov Med. 2019; 27: 125-138.

31. Batool M, Hillhouse AE, Ionov Y, Kochan KJ, Mohebbi F, et al. New Zealand White Rabbits Effectively Clear Borrelia burgdorferi B31 despite the Bacterium's Functional vlsE Antigenic Variation System. Infect Immun. 2019; 87.

32. Rogovskyy AS, Caoili SEC, Ionov Y, Piontkivska H, Skums P, et al. Delineating Surface Epitopes of Lyme Disease Pathogen Targeted by Highly Protective Antibodies of New Zealand White Rabbits. Infect Immun. 2019; 87.

33. Savić S, Vidić B, Lazić S, Lako B, Potkonjak A, et al. Borrelia burgdorferi in ticks and dogs in the province of Vojvodina, Serbia. Parasite. 2010; 17: 357-361.

34. Straubinger RK. PCR-Based quantification of Borrelia burgdorferi organisms in canine tissues over a 500-Day postinfection period. J Clin Microbiol. 2000; 38: 2191-2199.

35. Embers ME, Barthold SW, Borda JT, Bowers L, Doyle L, et al. Per-

sistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. PLoS One. 2012; 7: e29914.

36. Burgess EC. Borrelia burgdorferi infection in Wisconsin horses and cows. Ann N Y Acad Sci. 1988; 539: 235-243.

37. Burgess EC, Gendron-Fitzpatrick A, Wright WO. Arthritis and systemic disease caused by Borrelia burgdorferi infection in a cow. J Am Vet Med Assoc. 1987; 191: 1468-1470.

38. Marques A, Telford SR, Turk SP, Chung E, Williams C, et al. Xenodiagnosis to detect Borrelia burgdorferi infection: A first-in-human study. Clin Infect Dis. 2014; 58: 937-945.

39. Danzer H, Glaesner J, Baerenwaldt A, Reitinger C, Lux A, et al. Human Fcγ-receptor IIb modulates pathogen-specific versus self-reactive antibody responses in lyme arthritis. Elife. 2020; 9.

40. Fan Y, Yu D, Yao YG. Tree shrew database (TreeshrewDB): A genomic knowledge base for the Chinese tree shrew. Sci Rep. 2014; 4: 7145.

41. Whiteside SK, Snook JP, Ma Y, Sonderegger FL, Fisher C, et al. IL-10 Deficiency Reveals a Role for TLR2-Dependent Bystander Activation of T Cells in Lyme Arthritis. J Immunol. 2018; 200: 1457-1470.

42. Straubinger RK, Straubinger AF, Summers BA, Jacobson RH. Status of Borrelia burgdorferi infection after antibiotic treatment and the effects of corticosteroids: An experimental study. J Infect Dis. 2000; 181: 1069-1081.

43. Hodzic E, Feng S, Holden K, Freet KJ, Barthold SW. Persistence of Borrelia burgdorferi following antibiotic treatment in mice. Antimicrob Agents Chemother. 2008; 52: 1728-1736.

44. Barthold SW, Hodzic E, Imai DM, Feng S, Yang X, et al. Ineffectiveness of tigecycline against persistent Borrelia burgdorferi. Antimicrob Agents Chemother. 2010; 54: 643-651.

45. Feder HM, Johnson BJ, O'Connell S, Shapiro ED, Steere AC, et al. A critical appraisal of "chronic Lyme disease". New Engl J Med. 2007; 357: 1422-1430.

46. Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. New Engl J Med. 2001; 345: 85-92.

47. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, et al. The clinical assessment, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2006; 43: 1089-1134.

48. Wormser GP, Schwartz I. Antibiotic treatment of animals infected with Borrelia burgdorferi. Clin Microbiol Rev. 2009; 22: 387-395.

49. Marques A. Chronic Lyme disease: A review. Infect Dis Clin North Am. 2008; 22: 341-360vii-viii.

50. Bryksin AV, Tomova A, Godfrey HP, Cabello FC. BmpA is a surface-exposed outer-membrane protein of Borrelia burgdorferi. FEMS Microbiol Lett. 2010; 309: 77-83.

51. Johnston YE, Duray PH, Steere AC, Kashgarian M, Buza J, et al. Lyme arthritis. Spirochetes found in synovial microangiopathic lesions. Am J Pathol. 1985; 118: 26-34.

52. Philipp MT, Johnson BJ. Animal models of Lyme disease: Pathogenesis and immunoprophylaxis. Trends Microbiol.1994; 2: 431-437.

53. Steere AC, Brinckerhoff CE, Miller DJ, Drinker H, Harris ED, et al.

Elevated levels of collagenase and prostaglandin E2 from synovium associated with erosion of cartilage and bone in a patient with chronic Lyme arthritis. Arthritis Rheum. 1980; 23: 591-599.

54. Li D, Wu M. Pattern recognition receptors in health and diseases. Signal Transduct Target Ther. 2021; 6: 291.

55. Wang G, Petzke MM, Iyer R, Wu H, Schwartz I. Pattern of pro-inflammatory cytokine induction in RAW264.7 mouse macrophages is identical for virulent and attenuated Borrelia burgdorferi. J Immunol. 2008; 180: 8306-8315.

56. Janský L, Reymanová P, Kopecký J. Dynamics of cytokine production in human peripheral blood mononuclear cells stimulated by LPS or infected by Borrelia. Physiol Res. 2003; 52: 593-598.

57. Hurtenbach U, Museteanu C, Gasser J, Schaible UE, Simon MM. Studies on early events of Borrelia burgdorferi-induced cytokine production in immunodeficient SCID mice by using a tissue chamber model for acute inflammation. Int J Exp Pathol. 1995; 76: 111-123.

58. Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B, et al. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. J Exp Med. 1991; 173: 771-774.

59. Strieter RM, Kasahara K, Allen R, Showell HJ, Standiford TJ, et al. Human neutrophils exhibit disparate chemotactic factor gene expression. Biochem Biophys Res Commun. 1990; 173: 725-730.

60. Zlotnik A, Yoshie O. Chemokines: A new classification system and their role in immunity. Immunity. 2000; 12: 121-127.

61. Tiveron MC, Cremer H. CXCL12/CXCR4 signalling in neuronal cell migration. Curr Opin Neurobiol. 2008; 18: 237-244.

62. Zhu B, Xu D, Deng X, Chen Q, Huang Y, et al. CXCL12 enhances human neural progenitor cell survival through a CXCR7- and CXCR4-mediated endocytotic signaling pathway. Stem Cells. 2012; 30: 2571-2583.

63. Brunn A, Utermöhlen O, Mihelcic M, Sánchez-Ruiz M, Carstov M, et al. Differential effects of CXCR4-CXCL12- and CXCR7-CXCL12-mediated immune reactions on murine P0106-125 -induced experimental autoimmune neuritis. Neuropathol Appl Neurobiol. 2013; 39: 772-787.

64. Harvey B, Li L, Konrad M, Knight H, Westmoreland S, et al. FRI0034 Adalimumab reduces CXCR4 expression during inflammatory arthritis and in fibroblast-like synoviocytes and osteoclasts under chronic TNF exposure. Ann Rheum Dis. 2017; 76: 492-492.

65. Peng L, Zhu N, Mao J, Huang L, Yang Y, et al. Expression levels of CXCR4 and CXCL12 in patients with rheumatoid arthritis and its correlation with disease activity. Exp Ther Med. 2020; 20: 1925-1934.

66. Sucur A, Jajic Z, Artukovic M, Matijasevic MI, Anic B, et al. Chemokine signals are crucial for enhanced homing and differentiation of circulating osteoclast progenitor cells. Arthrit Res Ther. 2017; 19: 142.

67. Bradley JR. TNF-mediated inflammatory disease. J Pathol. 2008; 214: 149-160.

68. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. New Engl J Med. 2001; 344: 907-916.

69. Chen G, Wang W, Meng S, Zhang L, Wang W, et al. CXC chemokine CXCL12 and its receptor CXCR4 in tree shrews (Tupaia belangeri): Structure, expression and function. PLoS One. 2014; 9: e98231.