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Protective and Arthritis-Resolving Activity in Sera of Mice Infected with *Borrelia burgdorferi*

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Transfer of immune serum from immunocompetent mice infected with *B. burgdorferi* protects mice against syringe challenge, and transfer of immune serum after infection is established induces arthritis resolution but does not clear infection or spirochetemia or resolve carditis. Immune serum had very-high-titer passive protective activity against syringe challenge but failed to protect mice against host-adapted spirochetes when they were challenged with infected tissue transplants. Mice were passively immunized at selected intervals relative to challenge inoculation with antisera to recombinant forms of an immunodominant region of flagellin, P39, and OspC (which are recognized by immune serum), but none provided protection or modified existing infection or disease. Results suggest that spirochetes within joints, but not in other tissues, are selectively vulnerable to immune serum and that immune serum appears to contain antibody against yet-to-be-identified antigens that may be selectively expressed in the context of joint tissue.

The clinical manifestations of Lyme disease are inexplicably intermittent and changing. Early symptoms are multisystemic and then undergo spontaneous resolution, with episodic recurrence [1]. This implies persistent infection, and there is direct evidence that *Borrelia burgdorferi* can persist in humans that have not benefited from effective antibiotic therapy [2–16]. Further evidence of persistent infection is the rising titer and expanding immunoblot reactivity of antibody to *B. burgdorferi* in patient sera [17, 18]. Despite persistent infection, patients mount a protective immune response to *B. burgdorferi*, as transfer of their sera confers protection to laboratory rodents against *B. burgdorferi* challenge [19, 20].

The laboratory mouse is a useful model for examining mechanisms of host immunity. Inoculation of mice by tick feeding or by syringe with low doses of spirochetes results in multisystemic infection, with subsequent evolution of carditis and polysynovitis (arthritis), which undergo resolution and recurrent bouts of exacerbation during the course of persistent infection [21, 22]. Resolution of arthritis is heralded by disappearance of visibly discernible spirochetes from joints, but the spirochetes reappear in sites of recurrent arthritis [22, 23].

In contrast, mice with severe combined immunodeficiency (SCID) do not undergo carditis resolution and their arthritis becomes progressively more severe, with exuberant proliferation of spirochetes in synovium but not at other sites [23]. Passive transfer of immune serum, but not of T-enriched lymphocytes, from actively infected immunocompetent mice to SCID mice will confer protection if administered at or within a few days of challenge infection of recipient SCID mice; it induces arthritis resolution but does not cure infection when transferred after arthritis has become ongoing [24–26]. Thus, mice mount effective immune responses to *B. burgdorferi* during persistent infection but are unable to eliminate existing infection.

Both protective and arthritis-resolving activity can be compartmentalized to serum, but there are myriad potential antigens that could evoke these antibody-mediated phenomena. There is a relatively strong antibody response to the proteins flagellin, P39 (BmpA), and OspC in both the early and late stages of infection [17, 18, 27–32]. As infection proceeds, antibody reactivity appears against a number of additional proteins with remarkable size variation [17, 18, 27, 28, 30, 31, 33], including lipoproteins, core proteins, flagellar proteins, heat shock proteins, and others [34–54]. Furthermore, a growing number of proteins have been discovered that seem to be expressed exclusively in vivo, including EppA [55], P21 [56], PG [45], and BbK2.10 [52].

The purpose of the present study was to investigate the phenomena of serum-mediated protective immunity and arthritis-resolving immunity and attempt to incriminate *B. burgdorferi* antigens likely to drive these responses.

Materials and Methods

Mice. Random-sex, 3- to 4-week-old SPF C3H/HeN (C3H) mice were purchased from the NCI Animal Production Program of Frederick Cancer Research Center (Frederick, MD), and C3H/Smn.CIcrHsd-*scid* (C3H-*scid*) mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis). B10.A^k-μKO (μMT) mice were obtained from a breeding colony at our

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institution. Mice were maintained in isolator cages (Lab Products, Maywood, NJ) and provided food (Agway, Syracuse, NY) and water ad libitum. They were killed with carbon dioxide gas and then cardiocentesis was performed.

B. burgdorferi. Most experiments utilized a cloned and pathogenic *B. burgdorferi* sensu stricto strain N40 [22]. *Borrelia afzelii* strain PKo (*B. burgdorferi* sensu lato) was cloned by 3× limiting dilution, as previously described [22]. Spirochetes were grown in modified Barbour-Stoenner-Kelly (BSK-II) medium [57] and enumerated with a Petroff-Hauser bacterial counting chamber.

Histology. Rear limbs and hearts were processed with standard histology techniques. Arthritis was evaluated in sagittal sections of both knee and tibiotarsal joints from each mouse. Tibiotarsal arthritis severity was scored on a scale of 1 (mild) to 3 (severe) [23, 24], and arthritis prevalence was assessed by the number of arthritic joints among the four joints examined from each mouse. Carditis was evaluated in sagittal sections of the heart through the aortic valve [21–23, 58].

Indirect immunohistochemistry was performed on arthritic joints from infected C3H-*scid* mice. Tissue sections were bonded to glass slides, trypsinized, incubated with 1:10 dilutions of primary sera (90-day immune serum; hyperimmune sera to flagellin, OspC, and P39; and normal mouse serum), and then labelled with biotinylated goat antibody to mouse IgG by the streptavidin-peroxidase method, as previously described [58]. All histosections were examined and scored blindly.

Serology. Hyperimmune sera to recombinant proteins were generated in mice by primary subcutaneous immunization with 20 μg of protein in 0.1 mL of complete Freund's adjuvant, followed by 2 biweekly subcutaneous boosters of 10 μg of protein in 0.1 mL of incomplete Freund's adjuvant. All hyperimmune sera had immunoblot reactivity against their specific proteins of *B. burgdorferi* and their recombinant antigens at dilutions of >1:100,000.

Immunoblots were prepared by transfer of *B. burgdorferi* N40 or recombinant proteins from a 15% acrylamide resolving gel and 3% stacking gel to nitrocellulose paper, as described previously [59]. Immune serum was generated in culture-positive mice after intradermal inoculation with 10^2 *B. burgdorferi* spirochetes, an input dose ($<10^4$ spirochetes) that is antigenically subliminal until subsequent generations of spirochetes replicate and disseminate [60, 61]. Thus, the antibody response in immune serum is reflective of antigens expressed in vivo. Immune sera (30- and 90-day) had *B. burgdorferi* immunoblot titers of ≥1:200.

Passive immunization. Hyperimmune sera or immune sera were administered subcutaneously at doses and intervals specified in each experiment. Mice were syringe-challenged intradermally on the dorsolateral thorax with N40 or PKo spirochetes in 0.1 mL of BSK-II medium, contralateral to the site of passively transferred serum. Infection of mice was assessed by culture of cardiac blood, spleen, urinary bladder, ear punch, and inoculation site specimens, as described elsewhere [22]. The challenge dose was 10^4 spirochetes, unless otherwise noted. Mice were necropsied at intervals specified in each experiment, and disease status was assessed by histology.

Plasmid construction and recombinant fusion protein purification. *B. burgdorferi* N40 *p39* and *ospC* genes and an immunodominant region of *flagellin* (*p41G*), were amplified by PCR from *B. burgdorferi* N40 genomic DNA. *P41G* represents the region of flagellin to which the majority of *B. burgdorferi*–specific flagellin antibodies bind [62]. Oligonucleotide primers for *p41G* corresponded to nucleotides 589–603 and 819–905 of the N40 *flagellin* gene [62]. Primers for *ospC* corresponded to 58–69 and 616–627 of the N40 *ospC* gene [63] and the PKo *ospC* gene [41], respectively. Primers for *p39* corresponded to nucleotides 55–72 and 997–1020 of B31 *p39* [64]. The *ospC* and *p39* PCR amplification products lacked the sequences encoding the hydrophobic, N-terminal leader region (amino acids 1–18) to enhance solubility of the recombinant proteins [65].

N40 DNA was denatured at 94°C for 1 minute, annealed at 67°C for 1 minute, and extended at 72°C for 1 minute; this was repeated for 30 cycles. Amplified genes were cloned in frame with the glutathione S-transferase (GT) gene into pMX, a pGEX-2T vector (Pharmacia, Piscataway, NJ) with a modified polylinker [66]. PCR-amplified DNA sequences were confirmed by sequencing.

DH5α cells containing the recombinant pMX plasmids were grown to an optical density of 600 (~2 hours) and induced with 1 mM of isopropyl thiogalactose (2 hours). Bacterial cells were centrifuged at 4,000 rpm for 20 minutes; pellets were washed with PBS and then dissolved in 1/10 volume of PBS with 1% Triton X-100 (Sigma, St. Louis). The mixtures were sonicated and centrifuged at 10,000 rpm. Coomassie blue–stained gels showed that the recombinant fusion proteins were soluble and in the supernatants. The supernatants containing the fusion proteins were loaded onto glutathione-Sepharose 4B columns (Pharmacia).

P41G was not cleaved from its GT fusion partner, but GT was removed from GT-OspC and GT-P39 by cleaving of the fusion proteins bound on the columns with thrombin [34], with use of 25 U of thrombin (Sigma) added to the columns at room temperature for 2 hours. OspC and P39 were eluted with a solution of 50 mM of Tris/2.5 mM of $CaCl_2$/150 mM of NaCl. Eluents were incubated with antithrombin beads (Sigma) for 2 hours at room temperature and then centrifuged at 13,000 rpm to remove thrombin. GT-P41G, OspC, and P39 were then dialyzed against PBS, three times overnight.

Statistics. Prevalence data among treatment groups were compared by Fisher's exact test and deemed to differ from those for controls at the 95% confidence level ($P < .05$). Arthritis was considered resolving in treatment groups if mean arthritis severity scores and standard deviations did not overlap with control group scores and if arthritis prevalence differed from that among controls at the 95% confidence level.

Results

Arthritis resolution induced by 90-day immune serum. Passive transfer of immune serum to actively infected SCID mice induced arthritis resolution but did not eliminate carditis, infection [24], or spirochetemia (authors' unpublished observations). To confirm this phenomenon, groups of four C3H-*scid* mice were treated with 50 μL of 90-day immune serum or normal mouse serum at 6-day intervals commencing on day 12 after inoculation and then necropsied on day 30 or 60. At both 30 and 60 days, SCID mice treated with immune serum had significantly less arthritis severity and prevalence than did SCID mice treated with normal mouse serum. All 16 mice at both intervals had carditis with infection of multiple tissues, and all 16 mice were spirochetemic.

Arthritis resolution with different sera. We next treated SCID mice with different types of serum: 30-day immune serum (when arthritis is beginning to resolve [21, 22]); 90-day immune serum (when arthritis has resolved [21, 22]); serum from 3-week-old uninfected pups suckling immune (infected 1 month prior to whelping) dams (containing only the IgG fraction of immune serum, since pups selectively absorb IgG); 60-day immune serum from μMT mice (serum from infected B-cell-deficient mice without antibody [67]); and normal mouse serum.

Groups of 4 C3H-*scid* mice (plus 3 mice for normal mouse serum; total, 19 mice) were treated with 50 μL of each test serum at 4-day intervals commencing on day 12 and then were necropsied on day 28 relative to syringe inoculation with *B. burgdorferi.* All mice treated with normal mouse serum and "immune" serum from infected μMT mice had active arthritis of equal severity. In mice treated with 30-day immune serum, 90-day immune serum, and serum from pups suckling immune dams, arthritis resolved. All mice (19 of 19) were culture-positive, all were spirochetemic, and all had active carditis, regardless of serum treatment.

Arthritis resolution in C3H mice. To determine if arthritis resolution could be invoked in immunocompetent mice by passive transfer of immune serum and to determine if the lack of effect on spirochetemia or carditis also occurs in immunocompetent mice, we treated groups of four C3H mice with 50 μL of 90-day immune serum or normal mouse serum on days 4, 8, and 12 relative to syringe inoculation with *B. burgdorferi* and then necropsied them on day 14. This abbreviated treatment schedule was devised because arthritis at later intervals would be influenced by the recipients' acquired immunity and be resolved. None of the mice treated with immune serum had arthritis, but all mice treated with normal mouse serum had arthritis. Carditis was present in all mice but was less severe in mice treated with immune serum. All mice were culture-positive, and 4 of 4 mice treated with immune serum and 2 of 4 mice treated with normal mouse serum were spirochetemic.

Protective and arthritis-resolving immunity in mice challenged with host-adapted spirochetes. Mice can be infected with host-adapted spirochetes by subcutaneous transplantation of ear-punch tissue from infected donor mice [61, 68]. We therefore sought to determine if immune serum would protect mice against host-adapted spirochetes through transplantation challenge and if arthritis induced by such spirochetes was susceptible to immune serum–mediated resolution.

Groups of five C3H mice were passively immunized with 50 μL of 90-day immune serum or normal mouse serum on days 0, 4, 8, and 12 relative to syringe-challenge inoculation with 10^3 spirochetes or subcutaneous transplantation of ear-punch tissue from donor mice that had been infected for 2 weeks (table 1). We chose the 10^3-spirochete syringe dose on the basis of the fact that donor ear-punch transplants contain approximately this number of spirochetes, according to quantitative PCR [61].

Mice were necropsied at 15 days. Immune serum prevented infection by syringe challenge but not infection by transplantation challenge. Arthritis induced by transplantation challenge was vulnerable to the arthritis-resolving effects of immune serum, but spirochetemia and carditis were unaffected. Remarkably, transplantation-challenged mice treated with normal mouse serum developed arthritis (but not carditis) of significantly greater prevalence and severity than that in syringe-challenged mice.

Protective and arthritis-resolving activity in immune serum. To assess the relative strength of protective activity in immune serum, groups of four C3H mice were treated with 50 μL of 90-day immune serum or normal mouse serum and then challenged with 10^4, 10^5, 10^6, or 10^7 spirochetes at the time of serum treatment. At 2 weeks, all 16 mice treated with immune serum, regardless of the challenge dose, were protected against infection. All mice treated with normal mouse serum (10^4-spirochete challenge dose) had multisystemic infection.

We next compared the relative protective effects of immune serum in adult vs. 1-week-old C3H mice. Two mice of each age were each passively immunized with 10 μL of serial 2-fold dilutions of immune serum (1:20 through 1:640 dilutions) and simultaneously syringe-challenged with 10^4 spirochetes. Adult mice were protected against infection with serum dilutions of $\leq$1:40, whereas infant mice were protected against infection with serum dilutions of $\leq$1:320.

Since infant mice provide greater sensitivity, they are better suited for testing small amounts of immune serum for biological effect. We next optimized an assay for arthritis resolution in infant mice. Groups of three infant mice were inoculated with 10^4 spirochetes and then treated with 10 μL or 50 μL of 30- or 90-day immune serum on day 6, days 6 and 10, or days 6, 10, and 12. Control mice were given 50 μL of normal mouse serum on days 6, 10, and 12 (table 2).

On day 14, as expected, all mice were infected and nearly all were spirochetemic, regardless of treatment. Mice treated with normal mouse serum all had arthritis in multiple joints. Ninety-day immune serum had a stronger arthritis-resolving effect than did 30-day immune serum.

Table 1. Protective and arthritis-resolving immunity in C3H mice treated with 90-day immune serum on days 0, 4, 8, and 12 relative to challenge inoculation by syringe or infected-tissue transplantation.

Serum, mode of challenge	Spirochetemia*	Culture, all sites*	Arthritis severity†	Arthritis prevalence‡	Carditis*
Immune					
Syringe	0/5	0/5	0	0	0/5
Transplantation	5/5	5/5	1.1 ± 0.4	3.8 ± 0.4	4/5
Normal					
Syringe	2/5	3/5	0	0.1 ± 0.1	3/5
Transplantation	5/5	5/5	3.0 ± 0	4.0 ± 0	5/5

* No. of mice positive/no. tested.
† Tibiotarsal arthritis severity score (mean ± SD).
‡ Prevalence of arthritis among the four joints examined per mouse (mean ± SD).

Implication of B. burgdorferi antigens responsible for antibody-mediated protective immunity and arthritis resolution. We next sought to identify an early interval of infection at which immune serum provided protective and arthritis-resolving activity but had immunoblot reactivity to the fewest possible antigens. Infant mice passively immunized with 0.1 mL of 2-week immune serum at time 0 relative to challenge with 10^4 spirochetes were protected, and mice passively immunized with 0.3 mL of immune serum on days 6, 8, and 10 were infected and spirochetemic, and their arthritis resolved. Immunoblot profiles of 2-week immune serum revealed strong reactivity to flagellin, P39, and OspC, which have been previously identified with recombinant proteins and antisera [60].

Table 2. Arthritis resolution in infant mice treated with 10 μL or 50 μL of 30- or 90-day immune serum at different intervals after infection with *B. burgdorferi* N40.

Serum, volume (μL)	Serum given on day(s):	Spirochetemia*	Culture, all sites*†	Arthritis‡
30-Day immune				
10	6	2/3	3/3	3/3
	6, 10	2/3	3/3	2/3
	6, 10, 12	3/3	3/3	3/3
50	6	3/3	3/3	1/3
	6, 10	3/3	3/3	0/3
	6, 10, 12	2/3	3/3	0/3
90-Day immune				
10	6	3/3	3/3	3/3
	6, 10	3/3	3/3	3/3
	6, 10, 12	3/3	3/3	1/3§
50	6	3/3	3/3	0/3
	6, 10	3/3	3/3	0/3
	6, 10, 12	2/3	3/3	0/3
Normal serum				
50	6, 10, 12	3/3	3/3	3/3

* No. of samples positive/no. of samples tested.
† Combined results of cultures of blood, urinary bladder, spleen, ear and/or inoculation site specimens.
‡ No. of mice positive/no. tested.
§ Arthritis was mild and involved only one of the four joints examined.

Therefore, N40-specific flagellin (P41G), P39, and OspC recombinant proteins and high-titer hyperimmune sera to each recombinant protein were generated. Groups of four SCID mice were passively immunized with 50 μL of undiluted hyperimmune sera to GT-P41G, P39, or OspC; hyperimmune serum to GT (immunogen control); 90-day immune serum (positive-effect control); or normal mouse serum (negative-effect control). Antisera to GT-P41G, P39, or OspC were tested in separate experiments, each including GT antiserum and 90-day immune serum control groups (table 3).

In each experiment, groups of four SCID mice were treated on days 0, 4, 8, and 12 with GT antiserum or 90-day immune serum (except in the GT-P41G experiment, in which groups of mice were treated with 90-day immune serum on day 0; days 4, 8, and 12; or days 8 and 12), and groups of four SCID mice were treated with antiserum to the test antigen (GT-P41G, P39, or OspC) on day 0; days 4, 8, and 12; or days 8 and 12.

At 15 days, none of the antisera specific for the recombinant proteins provided protection against syringe challenge, none modified existing infection (including spirochetemia), and none modified arthritis severity, arthritis prevalence, or carditis. In contrast, 90-day immune serum, when administered on days 0, 4, 8, and 12, was strongly protective. When 90-day serum was given on day 0 or days 4 and 8, it was protective or eliminated early infection, whereas when given on days 8 and 12, it selectively induced arthritis resolution without affecting carditis or eliminating infection or spirochetemia.

Protective and arthritis-resolving immunity in SCID mice passively immunized with PKo-OspC antiserum. Antiserum against *B. burgdorferi* sensu lato PKo-OspC is protective against PKo challenge [69], but our data indicated that antiserum to N40-OspC was not protective against N40 challenge. Therefore, we sought to determine the protective and arthritis-resolving potential of PKo-OspC antiserum in C3H mice (table 4). Groups of four C3H mice were treated with 50 μL of antiserum to PKo-OspC (immunoblot titer, >1:100,000) on day 0; days 4, 8, and 12; or days 8 and 12 relative to syringe-inoculation with 10^3 PKo spirochetes.

Table 3. Protective and arthritis-resolving effects of antiserum to GT, GT-P41G (flagellin), P39, and OspC, as well as those of 90-day immune serum, in SCID mice passively immunized at different intervals relative to infection with *B. burgdorferi* N40.

Antigen	Antiserum or serum treatment group	Treatment day(s)	Spirochetemia*	Culture, all sites*†	Arthritis‡	Carditis‡
GT-P41G	GT	0, 4, 8, 12	3/3	4/4	4/4	4/4
	GT-P41G	0	4/4	4/4	4/4	4/4
		4, 8, 12	4/4	4/4	4/4	4/4
		8, 12	4/4	4/4	4/4	3/3
	90-d serum	0	0/4	0/4	0/4	0/4
		4, 8, 12	0/4	1/4	0/4	1/4
		8, 12	3/4	4/4	0/4	3/4
P39	GT	0, 4, 8, 12	4/4	4/4	4/4	4/4
	P39	0	4/4	4/4	3/4	3/4
		4, 8, 12	4/4	4/4	4/4	4/4
		8, 12	4/4	4/4	4/4	4/4
	90-d serum	0, 4, 8, 12	0/4	0/4	0/4	0/4
OspC	GT	0, 4, 8, 12	4/4	4/4	4/4	4/4
	OspC	0	4/4	4/4	4/4	4/4
		4, 8, 12	4/4	4/4	4/4	4/4
		8, 12	3/3	3/3	3/3	3/3
	90-d serum	0, 4, 8, 12	0/4	0/4	0/4	0/4
None	Normal serum	8, 12	2/2	2/2	2/2	2/2

* No. of samples positive/no. of samples tested.
† Combined results of cultures of blood, urinary bladder, spleen, ear and/or inoculation site specimens.
‡ No. of mice positive/no. tested.

A control group was treated with normal mouse serum on days 0, 4, 8, and 12. On day 15, mice were protected against challenge inoculation when PKo-OspC antiserum was given at the time of PKo challenge. Mice treated with antiserum on days 4, 8, and 12 were infected but had no arthritis. Mice treated with antiserum on days 8 and 12 were infected and spirochetemic, and most had arthritis. Mice treated with normal mouse serum were all infected and spirochetemic, and all had arthritis and carditis.

PKo is less pathogenic than N40, so carditis was not present in all mice and arthritis was generally mild and not scored for comparison. Results indicated that OspC-PKo antiserum protected against infection but had no effect on arthritis or spirochetemia in mice with established infections (day 8).

Immunohistochemistry. Immune serum has been shown to label spirochetes in joint tissues of infected SCID mice by indirect immunohistochemistry, but hyperimmune serum to killed *B. burgdorferi* or OspA did not label spirochetes [24, 61]. We therefore performed immunohistochemistry on infected SCID mouse joints, comparing 90-day immune serum with antisera to flagellin, P39, OspC, and normal mouse serum. Immune serum labeled spirochetes strongly, antiserum to flagellin labeled spirochetes very weakly, and antisera to P39, OspC, and normal mouse serum did not label spirochetes. Spirochetes were labeled with immune serum only in arthritic joint tissue—not in adjacent subcutis, muscle, or other tissue that contained visible spirochetes [23].

Discussion

The cumulative data from multiple experiments with use of passively immunized SCID mice as well as adult and infant

Table 4. Protective and arthritis-resolving effects of antiserum to *B. burgdorferi* PKo-OspC in C3H mice passively immunized at different intervals relative to infection with *B. burgdorferi* PKo.

Treatment group	Treatment day(s)	Spirochetemia*	Culture, all sites*†	Arthritis‡	Carditis‡
Antiserum to OspC	0	0/4	0/4	0/4	0/4
	4, 8, 12	2/3	4/4	0/4	2/4
	8, 12	3/3	4/4	3/4	2/4
Normal serum	0, 4, 8, 12	4/4	4/4	4/4	3/4

* No. of samples positive/no. of samples tested.
† Combined results of cultures of blood, urinary bladder, spleen, ear and/or inoculation site specimens.
‡ No. of mice positive/no. tested.

C3H mice provide convincing evidence that immune serum is strongly protective and can eliminate infection during the first few days of infection. However, when immune serum is given after infection is established, it cannot eliminate infection, including spirochetemia, but it induces arthritis resolution. Furthermore, although immune serum induces arthritis resolution when given after infection, it does not affect carditis.

These observations suggest that host-adapted spirochetes, except within the context of joint tissue, are not vulnerable to the effects of immune serum. This was confirmed by showing that immune serum protected against syringe challenge but not against transplantation challenge with host-adapted spirochetes.

The selective effect of passively transferred immune serum on arthritis, but not on carditis, may be related to the very different character of disease found in these two organs. Arthritis in the mouse is dominated by neutrophils and fibrin [21], whereas carditis is dominated by macrophages [58, 70]. Resolution of carditis may involve T helper cell type 1–dominated cellular responses, but this requires further scrutiny [71]. It is also of interest that spirochetes infect a wide variety of other tissues without inflammation, despite the fact that spirochetes can be discerned with silver stain in these sites [21–23].

Having compartmentalized both protective and arthritis-resolving activity to immune serum, and specifically to IgG, we reasoned that we could incriminate the responsible immunogenic antigen by examining immunoblots prepared with *B. burgdorferi* antigen and reacted with immune serum from mice at the earliest interval of infection that involves protective and arthritis-resolving activity. Serum from mice infected for 2 weeks has such qualities: it has protective and arthritis-resolving activity and is reactive against a limited repertoire of flagellin, P39, and OspC.

We attempted to implicate these antigens by generating recombinant forms of each, but none elicited protective or arthritis-resolving immunity. Results must be interpreted with the caveat that recombinant proteins may not be appropriately immunogenic, although this has not been the case with OspA, OspB, OspC (PKo), or OspF [32, 72].

Our findings that antiserum to the immunodominant fragment of flagellin was not protective were in keeping with active immunization studies with full-length flagellin [72]. Our findings that OspC antiserum did not protect against N40 spirochetes did not agree with findings of other investigators, who used other isolates of *B. burgdorferi*. Immunization against recombinant OspC-PKo protected gerbils against *B. burgdorferi* strain PKo [69], and immunization with OspC-Son188 protected mice against *B. burgdorferi* Son188 [73].

Because of these discordant findings, we generated a recombinant PKo-OspC with an approach identical to that for OspC-N40. Antiserum to PKo-OspC effectively protected mice against challenge inoculation with PKo but did not influence arthritis after infection was established. This lends credence to the possibility that recombinant OspC contains appropriately immunogenic (protective) epitopes, but OspC is not the antigen targeted by immune serum responsible for arthritis resolution or protection against N40 spirochetes.

An explanation for our results is that immune serum contains antibody to unidentified in vivo–expressed antigens that are not visible on *B. burgdorferi* immunoblots. Such antigens are not present on *B. burgdorferi* immunoblots, which represent antigens expressed in culture, but recombinant forms of these proteins are reactive with infected mouse serum, indicating that they are priming an immune response in vivo. These proteins include EppA [55], BbK2.10 (an OspF homologue) [52], P21 (an OspE homologue) [56], and PG [45]. To date, none of these proteins have been found to induce protective immunity, but other in vivo–expressed proteins are likely to be identified.

B. burgdorferi undergoes significant shifts in its antigenic profile in different environments. For example, OspA is abundantly expressed on the surface of spirochetes in culture and in the midgut of unfed ticks [74–77]. When ticks begin to feed, OspA expression is reduced. Spirochetes that migrate to the salivary gland during feeding, and subsequently into the host, have downregulated OspA [76, 77].

Thus, mice immunized against OspA are protected against syringe- and tick-borne infection, but when OspA-immune mice are challenged with host-adapted spirochetes (by transfer of infected tissue containing spirochetes that have downregulated OspA), they develop disseminated infection and full-blown disease [61]. Likewise, OspC appears to be upregulated in the feeding tick [77–79] and within the host [80]. Although OspA and OspC are not the antigens responsible for selective arthritis resolution, they illustrate the dynamic changes in *B. burgdorferi* during adaptation to the host and within the feeding tick in preparation for entry into the host.

Regardless of the immunogenic protein(s) that elicit these antibody-mediated responses, a remarkable and consistent finding in these studies is the selective effect of immune serum upon arthritis but not on carditis, infection status, or spirochetemia in actively infected mice. Since arthritis resolution is heralded by disappearance of spirochetes from joint tissue [21–23], spirochetes may be differentially vulnerable to the effects of immune serum within the context of joint tissue but not other sites.

Lipoproteins of *B. burgdorferi* have been shown to activate macrophages, B cells, T cells, and inflammatory mediators [81–90], and the lipid moiety of lipoproteins can elicit strong inflammatory responses when injected intradermally into mice [91]. These observations present a paradox. How can a highly immunogenic and inflammation-stimulating organism evade host immunity and persist in a number of tissues without inflammation? It has been proposed that *B. burgdorferi* may evade host immunity by expressing only small amounts of nonimmunogenic proteins on its outer surface [92]. The lack of protective immunity against host-adapted spirochetes, delivered by tissue transplantation challenge, could be explained by this hypothesis.

B. burgdorferi may very well become nonimmunogenic, but why does it elicit remarkably strong protective and arthritis-resolving antibody responses during active infection? A possible explanation is differential expression of immunogenic antigens within the context of certain tissues, such as synovium. It is attractive to speculate that spirochetes within synovium express (for reasons yet to be understood) immunogenic antigens, probably surface lipoproteins, that elicit intense inflammation, but spirochetes are nonimmunogenic in other tissues that do not manifest lesions.

This would explain the severe inflammatory response in joint tissue but not other tissues of infected mice. Notably, we have shown that spirochetes within arthritic synovium of SCID mice label with immune serum by indirect immunohistochemistry but do not label with antiserum to OspC or P39. In other studies, we could not label spirochetes in synovium with OspA antiserum or hyperimmune serum to heat-killed, cultured spirochetes [24, 61].

Such differential expression of immunogenic antigens may render synovial populations of spirochetes vulnerable to immune-mediated elimination, but spirochetes in other tissues, including blood and host-adapted spirochetes introduced by tissue transplantation, may be antigenically inert. This circumstance could explain the peculiar kinetics of protective antibody titers during persistent infection, which peak at 30 days and then decline progressively over months of persistent infection [60]. This suggests that immunologic priming ceases, possibly after arthritis undergoes resolution. Much remains to be learned about the complexities of the pathogen interaction in Lyme disease.

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