Mice Lacking CD21 and CD35 Proteins Mount Effective Immune Responses against *Borrelia burgdorferi* Infection[▽]

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Received 5 December 2006/Returned for modification 2 January 2007/Accepted 25 January 2007

CD21/35^{-/-} mice, deficient in CD21 and CD35 (complement receptors 2 and 1, respectively), were infected with *Borrelia burgdorferi* to assess the role of these receptors in a chronic bacterial infection. Although CD21/35^{-/-} mice on both C57BL/6 and BALB/c backgrounds produced less *B. burgdorferi*-specific antibodies than did wild-type mice, spirochete levels and arthritis severity were similar.

Complement plays an important part in the clearance of bacteria. The decoration of pathogen antigens with complement fragments provides signals important for the opsonization of bacteria and induction of inflammatory responses. In the mouse, CD21 and CD35 (complement receptors 2 and 1, respectively; CD21/35) are spliced products from the *Cr2* gene and serve as receptors for complement breakdown products. On B cells, CD21/35 functions as the B-cell coreceptor and augments B-cell signaling (5). Mice lacking CD21/35 (CD21/35^{-/-}) mount decreased antibody responses to T-cell-dependent and T-cell-independent antigens, with an impairment in the immunoglobulin G3 (IgG3)-specific antibody response (5). Consequently, CD21/35^{-/-} mice were more susceptible to lethal *Streptococcus pneumoniae* infection (6). These studies reveal an important role for CD21/35 in protective antibody responses during acute bacterial infection; however, the role of these proteins during a chronic bacterial infection has not been addressed.

Infection of mice with the spirochete *Borrelia burgdorferi* provides an excellent model for understanding host-pathogen interactions involved in chronic infection. Arthritis caused by

persistent infection of the spirochete within joint tissue appears to result from the sustained inflammatory challenges to the host from the bacterial components (18). Bacterium-specific antibodies produced during infection are not causative of or protective against acute Lyme arthritis in mice; however, they are important for controlling spirochete growth and resolution of disease (2, 14).

B. burgdorferi has evolved mechanisms to evade the host immune response, including resistance to host complement. *B. burgdorferi* is able to bind host complement regulatory proteins factor H and factor H-like 1, which permits the bacteria to evade complement-mediated killing (1, 4, 7–9, 13, 17). Additionally, higher spirochete levels and generally higher histopathology scores are found in joint tissue of C3^{-/-} mice (10). In these mice, anti-*B. burgdorferi* IgG levels were reduced compared to those in the wild type. These data suggest a role for complement proteins and their receptors in the immune response against *B. burgdorferi*.

In this study, we used *B. burgdorferi* infection to assess the role of complement receptor proteins CD21 and CD35 in a chronic bacterial infection. CD21/35^{-/-} mice were generated

TABLE 1. Effect of CD21/35 deficiency on *B. burgdorferi*-specific isotype distribution

Genotype ^a	Level (μg/ml) of <i>B. burgdorferi</i> -specific:				
	IgG	IgM	IgG1	IgG2b	IgG3
C57BL/6 WT	113.9 ± 26.4	41.0 ± 32.1	2.8 ± 1.4	92.8 ± 16.4	14.0 ± 8.1
C57BL/6 CD21/35 ^{-/-}	77.6 ± 26.3	56.8 ± 48.6	2.2 ± 1.8	59.4 ± 19.5 ^b	3.1 ± 1.6 ^b
C3H	232.9 ± 35.6	22.9 ± 20.6	27.1 ± 10.6	105.6 ± 1.4	4.4 ± 4.3
BALB/c WT	307.8 ± 18.6	23.7 ± 11.4	14.7 ± 1.8	897.8 ± 7.7	25.3 ± 10.7
BALB/c CD21/35 ^{-/-}	443.4 ± 15.4	51.0 ± 12.0 ^c	16.4 ± 5.2	417.5 ± 25.4 ^c	13.2 ± 4.6
C3H	772.2 ± 65.6	34.2 ± 11.3	7.0 ± 5.5	278.3 ± 38.4	15.5 ± 2.1

^a Number of mice per group = 5. WT, wild type.

^b Significantly different from wild-type C57BL/6 mice (*P* < 0.05) by Student's *t* test.

^c Significantly different from wild-type BALB/c mice (*P* < 0.05) by Student's *t* test.

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[▽] Published ahead of print on 5 February 2007.

FIG. 1. Diversity of antigens recognized by IgG and IgG3 in serum from infected CD21/35^{-/-} mice. Western blot analysis of IgG (A) and IgG3 (B) antibodies directed against *B. burgdorferi* in sera diluted to 1:200 from infected BALB/c and CD21/35^{-/-} mice sacrificed at 2 weeks or 4 weeks postinfection or in uninfected control sera. Similar results observed on a C57BL/6 background. OspA was detected with monoclonal OspA around 25 kDa, as indicated by prestained protein markers. OspC was detected with rabbit polyclonal OspC around 40 and 19 kDa. WT, wild type; Un-Inf, uninfected. The values on the left are molecular sizes in kilodaltons.

as previously described (6) and used at the 10th generation backcross to both C57BL/6 and BALB/c mice.

To assess the role of complement receptor proteins CD21 and CD35 in *B. burgdorferi*-specific antibody production, wild-type and CD21/35^{-/-} animals on both the BALB/c and C57BL/6 backgrounds were infected intradermally with a cloned N40 isolate of *B. burgdorferi* that had been passaged three times (provided by S. Barthold, University of California, Davis). To achieve equivalent disease severity on both backgrounds, mice on the BALB/c background received 2×10^4 bacteria, whereas C57BL/6 mice received 2×10^3 bacteria (12). C3H mice were infected with 2×10^3 bacteria as positive controls for infection. Bacterium-specific and total antibody titers were measured (12) at 2 and 4 weeks postinfection. *B. burgdorferi*-specific IgG3 antibodies were significantly decreased at 4 weeks postinfection in CD21/35^{-/-} animals on the C57BL/6 background, whereas a less drastic decrease was observed on the BALB/c background (Table 1). CD21/35^{-/-} mice produced increased bacterium-specific IgM antibodies on the BALB/c background. Knockout (KO) animals on both strain backgrounds produced significantly less *B. burgdorferi*-specific IgG2b (Table 1). Collectively, CD21/35^{-/-} mice had varied abilities to produce bacterium-specific antibodies im-

FIG. 2. Spirochete levels in joint tissue of infected CD21/35^{-/-} mice. *B. burgdorferi* DNA levels in the joints of infected C57BL/6 and CD21/35^{-/-} mice 4 weeks postinfection were assessed by quantitative PCR. Similar spirochete levels were observed in BALB/c and CD21/35^{-/-} mice, but they were lower than those in infected C57BL/6 or C3H animals.

TABLE 2. Ankle pathology in CD21/35^{-/-} mice infected with *B. burgdorferi*

Genotype ^a	Δ Ankle measurement (mm) ^b	Overall lesion ^c	Inflammation		Sheath thickness	Reactive repair	Total score
			Neutrophil	Mononuclear			
C57BL/6 WT	0.3 $\pm$ 0.2	1.4 $\pm$ 0.5	1.4 $\pm$ 0.9	0.6 $\pm$ 0.5	1.4 $\pm$ 0.9	0.6 $\pm$ 0.5	5.4 $\pm$ 2.7
C57BL/6 CD21/35 ^{-/-}	0.1 $\pm$ 0.1	0.6 $\pm$ 0.9	0.6 $\pm$ 0.9	0.4 $\pm$ 0.5	0.6 $\pm$ 0.9	0.0 $\pm$ 0.0	2.2 $\pm$ 3.2
C3H	0.9 $\pm$ 0.2	3.0 $\pm$ 1.0	2.6 $\pm$ 0.9	0.4 $\pm$ 0.5	3.2 $\pm$ 0.4	1.8 $\pm$ 0.8	11 $\pm$ 2.0
Uninfected WT	0.0 $\pm$ 0.0	0.4 $\pm$ 0.6	0.2 $\pm$ 0.5	0.2 $\pm$ 0.45	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.8 $\pm$ 1.1
Uninfected KO	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
BALB/c WT	0.4 $\pm$ 0.1	1.8 $\pm$ 1.3	0.6 $\pm$ 0.5	0.0 $\pm$ 0.0	1.4 $\pm$ 1.1	1.6 $\pm$ 1.1	5.4 $\pm$ 3.4
BALB/c CD21/35 ^{-/-}	0.3 $\pm$ 0.2	1.8 $\pm$ 1.6	1.0 $\pm$ 0.7	0.0 $\pm$ 0.0	1.0 $\pm$ 1.0	1.4 $\pm$ 1.9	5.2 $\pm$ 5.0
C3H	0.8 $\pm$ 0.3	2.4 $\pm$ 0.9	1.6 $\pm$ 0.5	0.0 $\pm$ 0.0	1.8 $\pm$ 0.8	1.4 $\pm$ 1.1	7.2 $\pm$ 2.8
Uninfected WT	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Uninfected KO	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

^a Number of mice per group = 5. WT, wild type.^b Rear ankle measurements reflect an increase in ankle size after 4 weeks of infection.^c Overall histopathology was scored in a blinded manner on a scale of 0 (no lesion) to 5 (severe lesion).

portant for immediate (IgM and IgG3) and long-term (IgG2b) protection. Total isotype-specific antibody titers in infected CD21/35^{-/-} mice were similar to those of infected wild-type mice and increased greater than twofold relative to those of uninfected mice (data not shown). This demonstrates that the CD21/35^{-/-} mice do not have an overall defect in the production of Ig against *B. burgdorferi*.

The decoration of bacteria with complement fragments provides signals for the opsonization, lysis, and clearance of the bacteria. To determine if the antigens recognized by CD21/35^{-/-} mice were altered relative to wild-type mice, Western blot analysis was performed comparing the antigens recognized by sera (3) from infected CD21/35^{-/-} mice and wild-type controls. IgG and IgG3 in sera from CD21/35^{-/-} and wild-type mice recognized similar proteins in *B. burgdorferi* lysates (Fig. 1). Sera from infected CD21/35^{-/-} and wild-type mice were also analyzed for antibody against the C6 peptide of the VlsE variable lipoprotein of *B. burgdorferi*, which has been used diagnostically for Lyme borreliosis in humans (11). There were no significant differences in anti-C6 peptide antibody levels between wild-type and CD21/35-deficient mice on both the C57BL/6 and BALB/c backgrounds (data not shown). In brief, CD21/35^{-/-} mice appear to recognize and mount antibody responses against a variety of *B. burgdorferi* antigens, including lipoproteins, albeit producing lower levels of *B. burgdorferi*-specific antibodies.

Bacterium-specific antibodies produced during infection play a role in controlling bacterial numbers and resolution of disease (14). To consider the effect of decreased *B. burgdorferi*-specific antibody responses in the CD21/35^{-/-} mouse, spirochete numbers and arthritis severity of the rear ankle joints of infected animals were assessed by quantitative PCR as described in detail in references 15 and 19. Deficiency in CD21/35 did not have an effect on *B. burgdorferi* numbers in ankle tissue 4 weeks postinfection (Fig. 2), indicating that the antibodies produced by a CD21/35^{-/-} animal are sufficient to control spirochete numbers. Spirochete numbers in the joint tissue of BALB/c mice correlates with arthritis severity (12). Arthritis severity within the ankle joints of infected mice did not differ significantly between wild-type and CD21/35-defi-

cient mice as seen by ankle swelling and histological analysis (Table 2).

Converse to the acute bacterial infection with *S. pneumoniae*, CD21/35^{-/-} mice demonstrated the ability to control *B. burgdorferi* infection to wild-type levels regardless of an altered repertoire of antibodies against the bacteria. This may be best explained by the role of antibodies in protecting the host against bacterial infections. IgG3 is the major mouse IgG isotype produced in response to T-cell-independent type 2 antigens like pneumococcal polysaccharides (16). Conversely, the immune response against *Borrelia* antigens uses both innate antibodies, like IgG3 and IgM, and those isotypes which require T-cell help, like IgG2a/b (14). Complement proteins may be more critical adjuvants for immune responses against *S. pneumoniae* polysaccharides than *B. burgdorferi* lipoproteins. The evasion of host complement activation by the more recently described *B. burgdorferi* CRASP proteins may be more critical for bacterial survival during the initial infection. Complement receptors CD21/35, on the other hand, would have a more significant role in complement-mediated immune responses to the bacteria within the splenic environment, like antibody production. The data presented here suggest that complement receptors CD21 and CD35 do not have a direct role in regulating spirochete numbers, and the ability of CD21/35^{-/-} mice to control *B. burgdorferi* infection suggests that *B. burgdorferi* does not have an advantage in a host lacking complement receptors 1 and 2.

This work was supported by U.S. Public Service grants AI-24158 to J.H.W. and AI-32223 to J.J.W. from the National Institutes of Health.

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Editor: W. A. Petri, Jr.