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Borrelia burgdorferi Infection and Immunity in Mice Deficient in the Fifth Component of Complement

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When immunocompetent mice are inoculated with *Borrelia burgdorferi*, they develop acute arthritis and carditis that undergo spontaneous regression despite the persistence of infection. Specific T- and/or B-cell immunity appears to be necessary for resolution of disease manifestations. Humoral immune responses to *B. burgdorferi* are also important in prevention of *B. burgdorferi* infection, in that passive transfer of immune sera or protective monoclonal antibodies prevents the spirochete from establishing infection. It has previously been suggested that complement is necessary for effective antibody-mediated host responses against *B. burgdorferi*. To investigate the role of complement in the pathogenesis and prevention of Lyme disease, we compared the responses to *B. burgdorferi* challenge inoculation of mice genetically deficient in the fifth component of complement (C5) with those of C5-sufficient mice. All C5-deficient strains tested were susceptible to *B. burgdorferi* infection, and disease manifestations underwent regression in a similar time-course to those of complement-sufficient mice. Moreover, passive immunization of C5-deficient mice with either immune rabbit sera or neutralizing monoclonal antibody protected them from challenge infection. These results demonstrate that the expression of Lyme disease is not altered in mice deficient in C5 and that C5-mediated complement activation is not necessary for antibody-mediated protection from infection.

Lyme disease (LD) is a multisystem disorder caused by the tick-borne spirochete *Borrelia burgdorferi* (14, 20, 21). In humans, infection with *B. burgdorferi* may result in skin rash, arthritis, carditis, and neurologic abnormalities. We have previously described a model for LD in immunocompetent mice which shares many of the clinical and histologic features of human LD (1, 3, 5, 6). All strains of mice are susceptible to *B. burgdorferi* infection, although disease severity varies among genotypes (1, 3, 5, 6). As in human LD, mice infected with *B. burgdorferi* develop acute arthritis and carditis that undergo spontaneous resolution despite persistence of infection. The specific components of host immunity that contribute to disease manifestations and their resolution are poorly understood. Experiments in severe combined immunodeficient (SCID) mice, which lack both T and B cells, have shown that disease manifestations occur despite these immune abnormalities, indicating that neither T cells nor B cells are necessary for development of disease (6, 19). In the SCID mouse, however, arthritis and carditis persist, suggesting that specific T- and/or B-cell immunity is required for disease regression.

The humoral immune response to *B. burgdorferi* is important in prevention of infection. Both passive transfer of immune sera to naive mice and active immunization of mice with *B. burgdorferi* prevent infection after challenge inoculation with *B. burgdorferi* (8, 18, 19). The presence of neutralizing antibodies to the outer surface protein A (OspA) of *B. burgdorferi* is sufficient to protect mice from *B. burgdorferi* infection. Vaccination of mice with recombinant OspA or passive immunization with the murine monoclonal antibody (MAb) VIIIC3.78, which binds a conformational epitope within the carboxyl half of the OspA protein (amino acids 133 through 273), protects mice from challenge infection (8, 17). The protective MAb VIIIC3.78 is an immunoglobulin G3 (IgG3) antibody that binds on immunoblots to the same region of OspA as an IgG1 MAb that is nonprotective (17). Although the epitopes recognized by these MAbs may be different, the fact that the protective MAb bears an IgG isotype capable of activating complement has led to speculation that complement may be necessary for antibody-mediated protection (17). This notion is supported by in vitro studies (12) suggesting that the bactericidal effect of *B. burgdorferi*-specific antibody is due to antibody-mediated complement activation. More recently, however, it has been proposed that the role of specific antibody is to increase the efficiency of the lytic activity of the C5b-9 membrane attack complex on *B. burgdorferi* (13).

The present study was designed to evaluate whether deficiency in C5-dependent complement activation affects the natural course of LD in mice or the ability of specific antibody to protect mice from *B. burgdorferi* challenge inoculation. The results reported herein demonstrate that both disease regression and protective immunity against Lyme disease in mice do not require complement activities dependent upon C5.

MATERIALS AND METHODS

Mice. Three-week-old mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The following strains of C5-deficient mice were used: A/J, AKR/J, B10.D2/oSnJ, DBA/2J, and SWR/J. C5 sufficiency or deficiency was determined by the vendor by using a sensitive hemolytic test measuring the ability of mouse sera to promote antibody-mediated lysis of sheep erythrocytes (11, 15). C5-sufficient control strains included B10.D2/nSnJ (congeneic with

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TABLE 1. Culture and histopathology results of C5-deficient and -sufficient strains of mice infected with *B. burgdorferi*[a]

Strain[b]	Carditis	Arthritis incidence	Arthritis severity	Culture
2 wk				
A/J (−)	3/3	4/4	2.0 ± 0 (93)	4/4
AKR/J (−)	3/3	4/4	2.3 ± 0.5 (100)	4/4
DBA/2J (−)	3/3	4/4	1.0 ± 0 (88)	3/3
SWR/J (−)	3/3	3/4	1.3 ± 1.0 (58)	4/4
C3H/HeJ (+)	2/2	2/2	2.0 ± 0 (88)	2/2
4 wk				
A/J (−)	4/4	4/4	1.5 ± 0.6 (69)	4/4
AKR/J (−)	4/4	4/4	2.8 ± 0.5 (88)	4/4
DBA/2J (−)	4/4	3/4	1.0 ± 0.8 (31)	3/3
SWR/J (−)	2/4	4/4	2.5 ± 0.6 (69)	4/4
C3H/HeJ (+)	2/2	2/2	3.0 ± 0 (50)	2/2
12 wk				
AKR/J (−)	3/6	2/6	0.3 ± 0.5 (11)	4/4
DBA/2J (−)	1/1	0/4	0	3/3
SWR/J (−)	2/5	5/5	1.2 ± 0.4 (44)	4/4
C3H/HeJ (+)	1/3	0/3	0	2/2

[a] Results for carditis and arthritis incidence are reported as number of positive mice over number of mice examined. The denominators vary because of the unavailability of some samples. Arthritis severity is reported as the average of the highest-scoring joint per mouse ± standard deviation. The numbers in parentheses indicate the percentage of all joints (knees and tibiotarsi) affected.

[b] −, C5 deficient; +, C5 sufficient.

B10.D2/oSnJ), DBA/1J, and C3H/HeJ (C3H). All mice were euthanized by carbon dioxide asphyxiation.

Bacterial strain and culture conditions. Low-passage *B. burgdorferi* N40, with previously proven infectivity and pathogenicity (1, 3, 5, 6), was used. Spirochetes were grown in modified Barbour-Stoenner-Kelly (BSK II) medium at 33°C in a standard air incubator as previously described (2).

Serum and MAb. Normal rabbit serum and immune sera from rabbits immunized with *B. burgdorferi* strain N40 were diluted 1:5 in phosphate-buffered saline (PBS). Immune rabbit sera contained *B. burgdorferi*-specific antibodies detectable on immunoblot with N40 lysate as substrate at dilutions up to 1:50,000. Passive transfer of 0.1 ml of the immune serum, diluted as much as 1:5,000, protected naive C3H mice against a standard intradermal challenge inoculum of 10^4 *B. burgdorferi* N40 spirochetes (7, 8). MAbs XH11.61 and VIIIC3.78 (both IgG3 isotypes) were derived from mice immunized with *B. burgdorferi* N40. XH11.61 binds the 41-kDa flagellin on immunoblots and, when passively transferred to naive mice, does not confer protection against *B. burgdorferi* challenge infection (9). VIIIC3.78 binds to a conformational epitope located within amino acids 133 to 273 of OspA and, in passive immunization experiments, is protective against *B. burgdorferi* challenge infection (17).

Passive immunization and challenge infection. Mice were infected with *B. burgdorferi* by intradermal inoculation with 10^4 spirochetes in BSK II medium. For passive immunization studies, mice received a single subcutaneous injection of serum diluted 1:5 in PBS or MAb hybridoma supernatant neat in a total volume of 0.1 ml 18 h prior to challenge inoculation. Two weeks after challenge inoculation, or at the indicated time points, mice were sacrificed and blood, ear punches, bladders, and spleens were cultured in BSK II medium for the presence of spirochetes. Hearts and joints (both knees and tibiotarsi) were fixed in formalin, embedded in paraffin and examined microscopically for evidence of disease. Arthritis severity was scored on a scale of 0 (no disease) to 3 (severe disease) as previously described (10).

RESULTS AND DISCUSSION

To determine the effect of C5 deficiency on the pathogenesis of Lyme disease in mice, we compared the outcomes of *B. burgdorferi* challenge inoculation of several C5-deficient strains of mice with that of C5-sufficient mice. We have previously demonstrated that *B. burgdorferi* infection of C3H mice, a C5-sufficient strain, results in arthritis and carditis that peak between 2 and 4 weeks of infection and then undergo spontaneous regression that is complete by 90 days (1, 3, 5, 6). All C5-deficient strains tested were susceptible to *B. burgdorferi* infection and disease, although the severity of disease manifestations varied among genotypes, a finding consistent with disease in complement-sufficient strains (Table 1). Similarly, arthritis (and carditis) underwent regression in the absence of C5, with fewer affected mice, fewer affected joints, and lower severity indices at 12 weeks compared with the 2- and 4-week time points (Table 1). All joints that were still positive at 12 weeks had features of resolution.

To determine whether biologic effects of complement activation involving C5 are necessary for antibody-mediated protection against infection, C5-sufficient and C5-deficient mice were passively immunized prior to challenge inoculation (Table 2). As expected, both C5-sufficient strains (B10.D2/nSnJ and DBA/1J) immunized with rabbit N40 antisera or the protective MAb VIIIC3.78 were resistant to *B. burgdorferi* challenge infection. Control mice immunized with either normal rabbit serum or the nonprotective MAb XH11.61 were susceptible to *B. burgdorferi* infection and developed histopathologic evidence of disease. C5-deficient

TABLE 2. Culture and histopathology results of *B. burgdorferi* challenge inoculation of C5-sufficient and -deficient mice after passive immunization with murine monoclonal and rabbit polyclonal antibodies[a]

Strain[b]	Antibody	Culture	Histopathology
Expt 1			
DBA/1J (+)	XH11.61 (IgG3, flagellin)	5/5	4/5
DBA/1J (+)	VIIIC3.78 (IgG3, OspA)	0/5	0/5
DBA/2J (−)	VIIIC3.78 (IgG3, OspA)	0/5	0/5
B10.D2/nSnJ (+)	Normal rabbit serum	5/5	5/5
B10.D2/nSnJ (+)	Rabbit N40 antisera	0/5	0/5
B10.D2/nSnJ (+)	XH11.61 (IgG3, flagellin)	5/5	5/5
B10.D2/nSnJ (+)	VIIIC3.78 (IgG3, OspA)	0/10	0/10
B10.D2/oSnJ (−)	Normal rabbit serum	5/5	5/5
B10.D2/oSnJ (−)	Rabbit N40 antisera	0/5	1/5
B10.D2/oSnJ (−)	XH11.61 (IgG3, flagellin)	5/5	5/5
B10.D2/oSnJ (−)	VIIIC3.78 (IgG3, OspA)	2/10	1/10
Expt 2			
B10.D2/nSnJ (+)	XH11.61 (IgG3, flagellin)	3/5	3/5
B10.D2/nSnJ (+)	VIIIC3.78 (IgG3, OspA)	0/5	0/5
B10.D2/oSnJ (−)	XH11.61 (IgG3, flagellin)	4/5	4/5
B10.D2/oSnJ (−)	VIIIC3.78 (IgG3, OspA)	0/5	0/5

[a] Results are expressed as the number of positive mice over the total number of mice in each experimental group.

[b] −, C5 deficient; +, C5 sufficient.

B10.D2/oSnJ and DBA/2J mice passively immunized with either immune rabbit serum or MAb VIIIC3.78 were fully protected from challenge infection with *B. burgdorferi*, as determined by culture and histopathology.

These results support the notion that activation of C5-dependent complement pathways is not necessary for either regression of LD manifestations, which may be dependent upon specific antibody, or for antibody-mediated resistance to *B. burgdorferi* infection in the mouse. Our studies utilized mice in which complement activity was deficient because of genetic defects resulting in the absence of C5. This essentially eliminates the concern that residual low levels of complement activity may be present, as might occur after complement depletion with cobra venom factor (22, 23). Moreover, it is unlikely that residual rabbit complement in the rabbit immune sera, which was heat-inactivated prior to use, could have been responsible for antibody-mediated protection because the protective MAb, which was generated in complement-free medium, also conferred resistance to *B. burgdorferi* infection. Two strains of mice (DBA and B10.D2) were used in passive immunization studies. The C5-sufficient DBA/1J mouse differs from its C5-deficient counterpart DBA/2J at chromosome 3 in the expression of the enzyme carbonic anhydrase and at chromosome 4 in the expression of glucose-6-phosphate dehydrogenase (Jackson Laboratory). It is unlikely, however, that these differences influenced the effect of C5 deficiency in humoral protection, in that similar results were obtained in the B10.D2/oSnJ mice, which differ from B10.D2/nSnJ mice only in the absence of C5.

The results of our studies with mice do not necessarily contradict those of Schmitz et al. (16), in which immune sera protected irradiated hamsters from *B. burgdorferi* infection by both complement-dependent and complement-independent mechanisms. Irradiated hamsters depleted of complement with cobra venom factor could be protected from challenge infection with 10^6 *B. burgdorferi* spirochetes by passive immunization with 3-week immune sera, but not with 1- or 10-week immune sera. Hamsters immunized with 1-week immune sera prior to challenge had delayed development of arthritis compared with hamsters receiving 10-week immune sera. It was suggested that the IgM antibodies present in the early sera may have been more effective in activating residual complement left after treatment with cobra venom factor than were the predominantly IgG antibodies in the late sera. In mice, IgM antibodies to *B. burgdorferi* can be detected at 4, 7, 14, and 30 days but not 90 days of *B. burgdorferi* infection (4). Although the mechanism(s) by which antibodies can kill *B. burgdorferi* is not known, pentameric IgM may be efficient at agglutinating spirochetes, thereby enhancing their elimination by nonspecific immune cells such as macrophages. When the amount of specific antibody is low relative to the number of spirochetes present, complement activation may augment humoral defenses against *B. burgdorferi*. In contrast, the IgG antibodies present in late sera may not be as effective in promoting agglutination of spirochetes, making the contribution of complement more apparent in protection at later time points. It is possible that the same is true in the hamster model of LD and that lower levels of protective IgG antibodies in 10-week immune sera require complement for full protection to be seen.

Although our results indicate activation of C5-dependent complement pathways is not necessary for either the pathogenesis of LD in mice or for antibody-mediated protection, complement activation does occur in response to *B. burgdorferi* (18). *B. burgdorferi* can directly activate both the classical and alternate complement pathways in the absence of antibody. The use of C5-deficient mice, which prevents C5-mediated responses and efficient formation of the membrane attack complex, does not preclude a role for other complement activation pathways in host resistance to *B. burgdorferi* infection. It is possible that other biologically active complement components (such as C1q, C3b, or C4b) enhance antibody or phagocytic cell bactericidal activity. Our studies do suggest, however, that in the presence of sufficient neutralizing antibody, complement activation involving C5 is not necessary for antibody-mediated protection against infection. This notion is supported by studies demonstrating that although antibodies can kill spirochetes by an action on C5b binding that leads to enhanced formation of the C5b-9 membrane attack complex, Fab fragments, which are incapable of activating complement, retain bactericidal activity, albeit at a lower efficiency than intact IgG (13). On the basis of these observations, we favor the view that antibodies mediate bactericidal activity by both complement-independent and complement-dependent pathways, with the latter mechanism serving to enhance the former.

In summary, the results reported herein represent the first detailed analysis of the effect of C5 deficiency on the course of LD and on passive transfer of resistance to *B. burgdorferi* infection in mice. Our results confirm that antibody-mediated bactericidal activity can occur in the absence of C5-dependent complement activation. Further studies are in progress to better define the mechanisms by which specific antibody mediates *B. burgdorferi* killing.

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