Generation of a Complement-Independent Bactericidal IgM against a Relapsing Fever Borrelia

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The spirochetemia of relapsing fever in mice is cleared by a complement-independent, polyclonal IgM response with reactivity to two prominent Ags of 20 and 35 kDa. In this study, we have dissected the polyclonal IgM Ab response against a relapsing fever spirochete to determine the specificity of its complement-independent bactericidal properties. Our experimental approach selectively generated an IgM murine mAb from the early specific immune response to a variable outer membrane protein. This IgM is bactericidal in the absence of complement and is part of the polyclonal Ab response that mediates the clearance of this bacterium from the blood. Purified monoclonal IgM caused direct structural damage to the outer membrane of the spirochete, in the absence of complement, and protected both B cell- and C5-deficient mice from challenge when administered passively. The direct, complement-independent, bactericidal activity of Abs is a critical mechanism of host defense against infection. The Journal of Immunology, 2004, 172: 1191–1197.

The tick-borne borrelioses include the etiological agents of both relapsing fever and Lyme disease. These spirochetes occur worldwide and share clinical characteristics that involve the nervous system, skin, joints, and heart (1, 2). However, Lyme disease lacks the marked and recurrent spirochetemia of relapsing fever (3). In both patients and murine models, the spirochetemia of relapsing fever is characterized by a large initial peak of organisms in the blood before their disappearance, subsequently followed by several smaller peaks of spirochetemia (3). Recurrence is due to antigenic variation of the organism (4).

Abs are indispensable in the host response to infection with *Borrelia*. The resolution of the first peak of spirochetemia in relapsing fever is mediated by a specific Ab response and is marked by the extremely rapid and complete clearance of organisms from the blood. We have previously identified an exclusively polyclonal IgM Ab response against the spirochete that is responsible for the elimination of organisms independent of either C3 or C5 (5). The importance of IgM in the resolution of spirochetemia is underscored by the persistence of spirochetes in the blood during the experimental infection of SCID. B cell-deficient, and secreting IgM-deficient mice (5–7). In contrast, the resolution of the initial peak of spirochetemia does not require the participation of T cells (7, 8).

These in vivo observations appear to be analogous to the previous characterization of C-independent bactericidal mAbs isolated following immunization with *Borrelia burgdorferi* or *Borrelia hermsii* (9–12). The IgG1κ murine mAb CB2 binds to outer surface protein (Osp) of *B. burgdorferi* and is directly bactericidal (10). Formation of the OspB-CB2 complex, dependent on a single lysine of OspB (Lys253) for binding, leads to the lysis of the outer membrane of the bacterium (11). CB2 is not a catalytic Ab, though its action is dependent on divalent cations and causes structural changes in OspB (11, 13). Significantly, the Fab of CB2 are also bactericidal, eliminating agglutination as the means of spirochete destruction by this Ab and underscoring its independence from complement (9–12). Other C-independent bactericidal Abs that recognize OspA, OspB, and p39 of *B. burgdorferi* have been reported (14–16), but the action of their Fab has not been evaluated.

Ab activity of this type is unique considering that destruction of bacteria is typically initiated by Ab-mediated complement fixation on the microbial surface, leading to lysis of the bacteria by the membrane attack complex of the complement cascade. Secondary mechanisms of bacterial clearance, such as opsonization, are mediated by the earlier complement components C1q and C3. In contrast, the direct, complement-independent lysis of bacteria by Abs represents a novel effector mechanism of the immune system with a clear and definitive role in immunity to tick-borne borrelioses and possibly to other bacteria as well.

We developed an approach for the generation of a C-independent bactericidal mAb from an experimentally infected mouse. The mAb was required to have the functional characteristics of the polyclonal Ab pool responsible for clearance of the spirochetemia. Specifically, the mAb should recognize a surface-exposed *Borrelia* membrane protein and be bactericidal in the absence of complement, similar to the bactericidal mAbs generated from immunization with *B. burgdorferi* and *B. hermsii* (9, 12). Our investigation of the adaptive immune response to an infection led to the generation of a monoclonal IgMκ against a 20-kDa outer membrane protein of a relapsing fever *Borrelia*. This mAb was specific for the infecting organism and was bactericidal in the absence of complement components. Furthermore, this mAb protected both B cell-deficient and C5-deficient mice from challenge when administered...
passively. This is the first instance of the isolation of a C-independent bactericidal mAb developed in response to an active infection. Our findings uncover a crucial in vivo role for an overlooked area of host defense and expand the array of the Ab effector functions.

Materials and Methods

Spirochetes and culture conditions

An uncultivable, patient-derived, virulent relapsing fever spirochete was maintained in the laboratory through mouse-to-mouse passage at the time of the first, and highest, peak of spirochetemia (17). *B. burgdorferi* strain B31 was originally isolated from Shelter Island, NY and grown in Barbour-Stoenner-Kelly (BSK) medium (Sigma-Aldrich, St. Louis, MO) without serum at 33°C (18). The relapsing fever spirochetes *B. hermsii*, obtained from R. C. Johnson (University of Minnesota, Minneapolis, MN), and *Borrelia crocidurae*, obtained from A. G. Barbour (University of California, Irvine, CA), were grown at 33°C in BSK medium supplemented with 6% rabbit serum.

Animal infection

Unless otherwise noted, the relapsing fever spirochetes used for inoculation were revived from frozen stocks and collected from a C3H/HeN donor mouse at peak spirochetemia (19). Before inoculation, spirochete-rich plasma was obtained by centrifugation of citrated whole blood at 800 × g for 15 min in a microcentrifuge (Eppendorf model 5415 C; Eppendorf, Germany). The plasma was centrifuged for 8 min at 8000 × g and the resulting spirochete pellet was washed twice with HBSS (Life Technologies, Carlsbad, CA). Mice were inoculated s.c. with 2 × 10⁵ spirochetes in 100 μl of HBSS. The progression of spirochetemia was monitored daily by dark-field microscopy and the first peak of spirochetemia was noted.

Animals

C3H/HeN and BALB/c mice were obtained from Taconic Farms (Germantown, NY). B cell-deficient mice (B6.129S2-IgH6) and wild-type (WT) controls (C3HBL/6), as well as C5-deficient (B10.D2/SN) mice and the congenic WT controls (B10.D2/sSnJ), were obtained from The Jackson Laboratory (Bar Harbor, ME) at 6 wk of age. C1q-deficient (C1qa−/−) mice were a gift from M. Botto (Imperial College, London, U.K.) and M. K. Ziegler (Harvard Medical School, Cambridge, MA) (20). Lewis rats, obtained from Charles River Breeding Laboratories (Wilmington, MA), were used to raise relapsing fever spirochetes as a source of *Borrelia* Ag. All complement-deficient and corresponding WT mice were housed in microisolator cages with free access to food and water. Microisolator cages of B cell-deficient and C1q-deficient mice were placed within laminar-flow biosafety cabinets. Animals were sacrificed by carbon dioxide asphyxiation followed by the collection of citrated whole blood via cardiac puncture. Animal procedures were done by protocols approved by the institutional review board.

mAb production

mAbs against the relapsing fever spirochetes were derived from the spleen of an infected BALB/c mouse immediately after the clearance of the first peak of spirochetemia and detection of *Borrelia*-specific Abs in the serum. Splenocytes from the mouse were fused to an Sp2/0-Ag-14 myeloma cell line (American Type Culture Collection, Manassas, VA) according to existing methodologies (22). Clones were screened for specific reactivity against both ELISA Ag by both ELISA and Western blot. One IgM clone, designated CB515, was selected and expanded for further analysis.

mAb purification

The hybridoma producing CB515 was grown in 60% SFM4Mab (HyClone, Logan, UT) and 40% DMEM supplemented with 100 μg/ml nonessential amino acids (Life Technologies, Grand Island, NY), 100 U/ml penicillin (Life Technologies), and 100 μg/ml streptomycin (Life Technologies). Serum-free medium was used to eliminate serum components from cell culture and to facilitate the purification of the mAb. Hybridomas were grown in the Integr CL-1000 in vitro ascites system (Integra Biosciences, Ijamsville, MD). Culture supernatants were concentrated using Centriprep YM-30 centrifugal filtration devices (Millipore, Billerica, MA) according to the manufacturer’s instructions. Buffer exchange ahead of chromatography was accomplished by dialyzing the concentrated hybridoma supernatant against PBS (pH 7.2) (Life Technologies) using a Slide-a-Lyzer cassette of Mr 10,000 cutoff (Pierce, Rockford, IL). Once dialyzed, the concentrated supernatant was passed through a 0.22-μm syringe filter and stored at 4°C.

Final purification of the monoclonal IgM by size exclusion chromatography utilized a prepacked Superose 6 HR10/30 column (Amersham Biosciences, Piscataway, NJ) in tandem with an AKTA fast protein liquid chromatography and fraction collector (Amersham Biosciences). An aliquot of concentrated, dialyzed, and filtered culture supernatant was added to the column and resolved at a rate of 0.1 ml/min. PBS was used as the isocratic buffer to maintain a physiological pH and ionic strength. Fractions were collected, assayed for purity by SDS-PAGE and Western blot, and quantified by a MicroBCA protein assay (Pierce).

ELISA

ELISA, using sonicated whole-cell relapsing fever spirochetes as the Ag (described below), was used to screen the initial supernatants of the hybridoma fusions. Briefly, 96-well polystyrene plates were coated by overnight incubation of 5 μg/ml Ag in a high pH (pH 9.6) carbonate buffer at 4°C. After incubation, the plates were blocked for 30 min in a buffer composed of PBS, 0.05% Tween 20 (Fisher, Pittsburgh, PA), 0.5% BSA (Sigma-Aldrich), and 0.02% NaN₃ (Sigma-Aldrich). Blocking was followed by three washes of wash buffer consisting of PBS, 0.05% Tween 20, and 0.02% NaN₃. Undiluted hybridoma supernatant was added to each well and allowed to incubate for 1 h at 37°C. Plates were washed dry, washed three times with wash buffer, and incubated for 1 h with alkaline phosphatase-conjugated goat anti-mouse IgM at 1 μg/ml (μ-chain specific) (Kirkegaard & Perry Laboratories, Gaithersburg, MD). After the incubation, the ELISA was developed with the addition of p-nitrophenyl phosphate (Sigma-Aldrich). OD was read with a Dynatech MR-580 Micro ELISA reader (Dynatech Laboratories, Alexandria, VA) at a wavelength of 410 nm.

SDS-PAGE, Western blot, and protein identification

Ag from *B. burgdorferi*, *B. hermsii*, and *B. crocidurae* was collected by centrifugation of cultures at 8000 × g for 8 min, washed with HBSS, and sonicated. Relapsing fever spirochetes were collected from the plasma of infected Lewis rats at the first peak of spirochetemia and processed as above. All *Borrelia* sonicates were electrophoresed on 12.5% SDS-PAGE gels under reducing conditions. Gels were stained for total protein by silver impregnation (Silver Stain Plus; Bio-Rad, Hercules, CA) or were transferred to nitrocellulose for immunoblotting.

For the screening of Ab-producing hybridomas, supernatants were diluted 1/10 in PBS containing 5% casein (Sigma-Aldrich) and allowed to incubate for 1 h. Alkaline phosphatase-conjugated goat anti-mouse IgM (μ-chain specific) were used as the secondary Ab at 1 μg/ml in PBS. L chain specificity of CB515 was determined by Western blot using secondary Abs specific for the κ or λ chain (Sigma-Aldrich). Immuneblots were developed through reaction with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Kirkegaard & Perry Laboratories).

Proteolytic degradation, HPLC separation, and matrix-assisted laser desorption ionization-time-of-flight mass spectroscopy were performed by the Stanford PAN Facility (Stanford University, Stanford, CA). Masses obtained from the mass spectroscopy analysis were subjected to database searches while internal peptide sequences were aligned using a protein-to-protein Basic Local Alignment Search Tool.

Two-dimensional electrophoresis

*Borrelia* Ag was reconstituted in a rehydration buffer containing 8 M urea (Roche, Indianapolis, IN), 1% 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (Sigma-Aldrich), 15 mM DTT (Roche), and 0.2% w/v Biolytes 3/10 (Bio-Rad). Rehydration of ReadyStrip IPG strips (Bio-Rad) of a 3–10 pH range with the rehydrating solution occurred overnight according to the manufacturer’s instructions. Isoelectric focusing was completed using the Protean IEF cell (Bio-Rad). The second dimension was resolved by SDS-PAGE. Gels were transferred to nitrocellulose for immunoblotting.

In vitro killing assay

Spirochetes were collected from the blood of C5-deficient mice at the first peak of spirochetemia by centrifugation of plasma at the secondary Ab, and dialyzed mAb, either CB515 or the irrelevant IgM control, was added at a concentration of 100 μg/ml and the reaction was allowed to proceed for 10 min at 33°C.
Transmission electron microscopy (TEM)

Pellets from the in vitro killing assay reaction were gently resuspended in 50 μl of PBS and allowed to adsorb to polyvinyl formal-coated grids (Ernest F. Fullam, Latham, NY) for 2 min. Grids were fixed in 1% glutaraldehyde (Sigma-Aldrich), washed twice in PBS and water, and stained for 25 s with 0.5% phosphotungstic acid. Images were captured using a JEOL JEM-1200EX II transmission electron microscope (JEOL USA, Peabody, MA) at an accelerating voltage of 80 kV.

Passive immunization and challenge

Mice were passively immunized daily with 50 μg of purified CB515 or 50 μg of an irrelevant murine monoclonal IgM (TEPC 183; Sigma-Aldrich) in PBS by i.p. injection. Immunization began 2 days before and ended 4 days after s.c. challenge with 2 × 10^4 relapsing fever spirochetes. Spirochetes used to infect the C5-deficient mice were collected from a C5-deficient mouse to avoid the transfer of endogenous C5. The daily passive immunization regimen was chosen to compensate for the deficiency in C1q. The daily passive immunization regimen was chosen to compensate for the deficiency in C1q. The daily passive immunization regimen was chosen to compensate for the deficiency in C1q.

Results

Complement component C1q is not required for the clearance of spirochetemia

Our previous work revealed that neither C3 nor C5 is required for the clearance of spirochetemia (5). However, these studies did not eliminate the role of opsonization and phagocytosis of organisms through the formation of an IgM-C1q complex. To address this, C1q-deficient mice were s.c. inoculated with 2 × 10^4 spirochetes and monitored for the progression of spirochetemia. Consistent with our previous work with animals deficient in later components of the complement cascade, a deficiency in C1q did not alter the course of spirochetemia (Fig. 1). C1q-deficient mice were able to clear the organisms from the blood and generated an Ab response to the spirochete (data not shown). Therefore, the presence of an intact classical pathway of complement activation is not required to resolve the spirochetemia of relapsing fever.

Generation of a mAb responsible for clearance of spirochetemia

To develop a mAb from the polyclonal Ab response that is critical for the rapid and complete clearance of spirochetemia, we inoculated a BALB/c mouse s.c. with 2 × 10^4 spirochetes. The course of spirochetemia was followed closely to the point of clearance. Immunoblots confirmed that a specific IgM response had been generated against the spirochetes at this time, as found previously (5). This specific IgM was directed against two immunodominant Ags migrating at 20 and 35 kDa. Clones derived from the fusion of splenocytes to a nonproducing fusion partner were exclusively IgM-secreting hybridomas. Upon subcloning, all clones were of the IgM class against an unknown 20-kDa Borrelia protein. Hybridomas producing IgM against the 35-kDa Ag were refractory to growth upon subcloning. One IgM clone, designated CB515, was expanded for further analysis.

CB515 targets a variable small protein

Previous complement-independent bactericidal mAbs have been shown to target surface-exposed Borrelia proteins. The reactivity of CB515 was specific for the relapsing fever spirochete that infected the BALB/c mouse from which the spleen was derived. Although generated early in infection, the IgM response against the relapsing fever Borrelia was specific for the infecting organism, resulting in an acquired immune response to the 20-kDa Ag, visible as a major band by silver stain (Fig. 2, top panel). CB515 did not cross-react with the Lyme disease spirochete B. burgdorferi or the relapsing fever spirochetes B. hermsii and B. crocidurae (Fig. 2, middle panel). Upon isoelectric focusing, the unknown 20-kDa Ag exhibited a trailing pattern characteristic of a posttranslationally modified protein (Fig. 2, bottom panel) (24, 25). Given the molecular mass and isoelectric focusing pattern of Ag recognized by CB515, as well as the knowledge that the major immunogenic proteins recognized by postpeak serum are borrelial variable proteins (4), we hypothesized that CB515 binds to a variable small protein.

Consistent with a lipid modification at the amino terminus, no signal was obtained by Edman degradation of the 20-kDa Ag band. To gain an internal sequence, Lys-C endoproteinase fragments of the 20-kDa Ag, resolved by SDS-PAGE, were separated by HPLC and subjected to both matrix-assisted laser desorption ionization-time-of-flight mass spectroscopy and Edman degradation. Mass spectroscopy analysis of the proteolytic degradation products resulted in the generation of 12 distinct fragments (data not shown).

![FIGURE 2](http://www.jimmunol.org/)

CB515 is specific for a 20-kDa lipoprotein. Top panel. Silver-stained SDS-PAGE of whole-cell lysates of an uncultivable relapsing fever Borrelia (A), B. burgdorferi (B), B. hermsii (C), and B. crocidurae (D). Middle panel. Corresponding Western blot showing specific reactivity of the monoclonal IgM (CB515) against a 20-kDa band in the uncultivable relapsing fever Borrelia. Bottom panel. Western blot with CB515 of two-dimensional electrophoresis of the relapsing fever Borrelia showing the trailing pattern of the 20-kDa Ag. Molecular mass, in kilodaltons, shown on left.

![FIGURE 1](http://www.jimmunol.org/)

C1q is not required to clear spirochetemia. Plotted is the mean (±SD) course of spirochetemia in C1q-deficient (n = 3) mice after s.c. inoculation of 2 × 10^4 Borrelia. Both C1q-deficient and WT mice (data not shown) cleared the spirochetemia rapidly after reaching peak, resulting in no visible spirochetes in the blood.
FIGURE 3. CB515 disrupts the bacterial outer membrane in the absence of complement. Negative-stained TEM of the relapsing fever spirochete after irrelevant IgM treatment (A) and after treatment with CB515 for 10 min (B–L). C, F, I, and L are higher magnifications of B, E, H, and K, respectively. Treatment of the spirochetes with CB515 leads to the destruction of the outer membrane and release of the periplasmic flagella. Scale bars are as follows: A, D, G, and K, bar = 1 μm; B, C, F, and L, bar = 200 nm; E, H, I, and J, bar = 500 nm.
A database search did not result in an identifiable match to any known protein. Upon Edman degradation, one internal peptide sequence was obtained from these resulting fragments: GLETTDNSDELK. The peptide was found to be identical to a sequence within variable major protein 24 of the B. hertsmii strain HS1 (GenBank accession no. AAA22964), a member of Borrelia outer membrane proteins responsible for the antigenic variation of the organism. Therefore, CB515 met the predefined criteria that the mAb targets a surface-exposed protein.

The C-independent bactericidal effect of CB515 leads to the destruction of the outer membrane

Incubation of purified CB515 with spirochetes, in the absence of complement, resulted in the destruction of the bacterial outer membrane. This destruction was evident in the ultrastructural changes to the spirochete as visualized by TEM of negative-stained, whole-cell spirochetes. Examination of the spirochetes treated with an irrelevant murine IgM revealed the characteristic spiral shape of the bacterium, an intact outer membrane, and the absence of expressed periplasmic flagella (Fig. 3A). Similar results were observed in the untreated controls (data not shown). Upon the addition of CB515, the direct bactericidal activity of the Ab is evident in the damage of the bacterial outer membrane. Representative examples of spirochete damage are shown in B through L of Fig. 3. As a result of outer membrane destruction, the direct bactericidal activity of Abs against Borrelia exposes the periplasmic flagella concurrent with spirochete killing (11). As seen in Fig. 3, CB515-treated spirochetes have lost the integrity of their outer membrane, resulting in the formation of membranous blebs similar to the CB2-mediated killing of B. burgdorferi (11). In a separate experiment, incubation of 2.7 10^8 spirochetes with 100 ȝg/ml CB515 resulted in a 77% reduction in spirochete numbers after 60 min compared with untreated controls. This in vitro killing by CB515 is representative of the polyclonal IgM response that mediated the complete clearance of the initial peak of spirochtemia in complement-deficient and WT mice (5), and thus met the requirements of our experimental approach.

Passive immunization with CB515 protects both C5-deficient and B cell-deficient mice from challenge

Passive immunization has consistently demonstrated a protective capability against infection with both Lyme disease and relapsing fever Borrelia (26–29). The protective capacity of a monoclonal, complement-dependent IgM against a relapsing fever spirochete has been measured in irradiated WT mice (30), and specific polyclonal IgM is sufficient to passively protect B cell-deficient mice (5). Given the efficacy of specific Ab to protect mice from challenge, a passive immunization regimen was completed to determine the protective capacity of this most recently developed bactericidal mAb.

As shown in Tables I and II, in both the C5-deficient and B cell-deficient mice, the administration of CB515 conferred protection against a challenge inoculation of 2 10^8 spirochetes. The massive spirochtemia previously shown to occur in the absence of IgM (5, 7) was reduced in B cell-deficient (B6.129S2-IgH6) mice administered CB515, but not in mice that received an irrelevant murine monoclonal IgM (Table I). Although the WT (C57BL/6) mice did not exhibit the same decrease in spirochtemia seen in the B cell-deficient mice, as would be expected, administration of CB515 delayed the onset of spirochtemia by 1 day (data not shown), consistent with protective activity. Thus, in both the B cell-deficient and WT mice, passive immunization with a C-independent bactericidal mAb exhibits a protective capacity upon challenge. In immunoblots, CB515 did not recognize the spirochetes that grew in the B cell-deficient mice that were passively immunized (data not shown). The lack of CB515 reactivity demonstrates the growth of escape mutants following passive immunization.

A more dramatic effect was seen upon administration of CB515 to both C5-deficient (B10.D2/oSnJ) and the congenic WT (B10.D2/mSnJ) mice. In these animals, passive immunization with CB515 resulted in the complete protection from challenge up to 10 days postinoculation (Table II). The total elimination of organisms from the C5-deficient mice, as opposed to the growth of escape mutants in B cell-deficient mice, may reflect the action of an incipient IgM response that worked synergistically with the passively administered Abs. Control animals left untreated developed a spirochtemia as expected. These results parallel the protection provided by polyclonal IgM in the B cell-deficient model (5).

**Discussion**

The clearance of the spirochtemia of relapsing fever is mediated by complement-independent bactericidal Abs. Mice with a single gene deficiency in C1q (this study), as well as those lacking C3 or C5 (5), do not exhibit any clearance defects. In contrast, mice unable to produce IgM are incapable of clearing the organisms

<p>| Table I. Passive immunization confers protection to B cell-deficient mice from challenge^a^ |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>B Cell-Deficient B6.129S2-IgH6 (n = 3)</th>
<th>B Cell WT C57BL/6 (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrelevant IgM</td>
<td>1.02  10^6  ±  2.08  10^7</td>
<td>5.39  10^7  ±  3.89  10^7</td>
</tr>
<tr>
<td>CB515</td>
<td>2.80  10^7  ±  6.71  10^6</td>
<td>2.80  10^7  ±  1.85  10^7</td>
</tr>
</tbody>
</table>

^a^ Mice were passively immunized daily with 50 ȝg of purified CB515 by i.p. injection beginning 2 days before and ending 4 days after s.c. challenge with 2 10^8 relapsing fever spirochetes. Shown is the mean ± SD of the spirochtemia at peak. Spirochtemia was measured for 10 days.

<p>| Table II. Passive immunization confers complete protection to C5-deficient mice from challenge^a^ |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>C5-Deficient B10.D2/oSnJ (n = 3)</th>
<th>C5 WT B10.D2/mSnJ (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>4.56  10^7  ±  3.94  10^6</td>
<td>1.46  10^7  ±  3.81  10^6</td>
</tr>
<tr>
<td>CB515</td>
<td>No spirochtemia</td>
<td>No spirochtemia</td>
</tr>
</tbody>
</table>

^a^ Mice were passively immunized daily with 50 ȝg of purified CB515 by i.p. injection beginning 2 days before and ending 4 days after s.c. challenge with 2 10^8 relapsing fever spirochetes. Shown is the mean ± SD of the spirochtemia at peak. Spirochtemia was measured for 10 days.
from the blood and, subsequently, develop a chronic blood infection (5–7). Previous studies have established the validity of spirochetemia destruction by C-independent bactericidal mAbs (9–12) and indirect evidence has supported the role of this type of Ab in vivo (5, 14, 31). However, the isolation of such an Ab from a specific immune response to an infection has not been achieved. Because of this, we undertook an experimental approach to generate a mAb of known specificity that had the C-independent bactericidal properties identical to the polyclonal response observed in both in vivo and in vitro experiments.

In this study, we developed a murine monoclonal IgMx (CB515) from the polyclonal Ab pool that mediates the rapid and complete clearance of spirochetemia. This monoclonal IgM is bactericidal in the absence of complement, resulted from the early, specific immune response against the infecting organism, and is directed against a 20-kDa outer membrane protein with sequence similarity to variable major protein 24 of B. hermsii. This mAb does not cross-react with B. burgdorferi or the relapsing fever spirochetes B. hermsii and B. crocidurae. Incubation of the spirochetes in an in vitro killing assay with purified CB515, in the absence of complement, results in structural damage to the spirochete that can be visualized by TEM. The direct bactericidal action of CB515 disrupts the outer membrane of the spirochete, with the subsequent exposure of periplasmic flagella. A similar disruption of the outer membrane of B. burgdorferi by CB2 (9) and another mAb (12) has been shown by thin-section TEM. Agglutination is not considered a means of spirochete destruction by this Ab as another monoclonal IgM, against B. burgdorferi, can agglutinate but is not bactericidal (25). Administration of CB515 can passively protect both B cell-deficient and C5-deficient mice from challenge.

Recent evidence has shown that Abs, as well as their Fab, are capable of generating reactive oxygen species that contribute to bactericidal activity, regardless of specificity (32). However, this bactericidal activity requires the presence of superoxide anion, presumably provided by the oxidative burst of a phagocyte, for bacterial killing in the absence of complement (33). Given that the CB515-mediated lysis of the spirochete occurs in the absence of phagocytes, it is unlikely that this is the mechanism of action. Furthermore, the incubation of spirochetes with an irrelevant murine mAb, TRPC 183, did not result in the lysis of the organism. However, we cannot preclude the necessity of specific binding, absent in the irrelevant IgM, to bring the reaction in close proximity with the bacterial outer membrane. Although we have demonstrated that complement-independent Abs can destroy Borrelia both in vitro and in vivo, this does not preclude that complement, when present, can participate in bactericidal mechanisms.

In this study, we successfully completed an experimental approach to generate a C-independent bactericidal mAb that has the same function as the in vivo polyclonal Ab response. This is the first demonstration of a directly bactericidal mAb isolated from the host response to an active infection. Our findings reveal a critical in vivo role for an overlooked Ab effector mechanism and point to the therapeutic potential of C-independent, bactericidal Abs. The role of Abs in defense against microorganisms has acquired increased significance in light of new and renewed microbial threats, both natural and man-made. The rapid proliferation of new infectious agents, the emergence of classical infections resistant to treatment, and the threat of biowarfare have fueled the need for new approaches to host defense. Multiple methods have been taken to use Abs against microorganisms, as evidenced in the re-emergence of Ab-based therapies, including passive immunization, postexposure prophylaxis, and immunotoxin treatment (34–38). The development of these treatments demands the re-evaluation of the way Abs mediate microbial infection as current depictions of Ab mechanisms are incomplete. In addition to complement activation, opsonization, toxin and viral neutralization, agglutination, Ab-directed cellular toxicity, and inhibition of microbial attachment, the direct bactericidal activity of Abs must be considered as a mechanism of host defense. Given the role of such bactericidal Abs in tick-borne borreliosis (9–12), as well as the ability of Abs to nonspecifically generate bactericidal products (32), the expanding repertoire of Ab function should also include direct action on the organism.

References


