Complement-Mediated Killing of *Borrelia garinii* —Bactericidal Activity of Wild Deer Serum

Emiko Isogai*1, Yuri Kamewaka2, Hiroshi Isogai3, Koichi Kimura4, Nobuhiro Fujii4, and Takeshi Nishikawa5

1Department of Preventive Dentistry, Health Sciences University of Hokkaido, Hokkaido 061-02, Japan, 2Health Center of Rumoi, Hokkaido 077, Japan, 3Center of Animal Experimentation, and 4Department of Microbiology, Sapporo Medical University, Sapporo, Hokkaido 060, Japan, 5Department of Dermatology, Hokkaido University School of Medicine, Sapporo, Hokkaido 060, Japan

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**Abstract:** The susceptibility of *Borrelia garinii* to fresh wild deer sera was determined by incubating strain SIKA2 at 10% serum concentration for 1 hr at 37°C in an in vitro bactericidal assay. Each serum showed bactericidal effects at various levels. The effect was dependent on the concentration of antibody to the spirochetes. Complement was essential in the bactericidal assay because the inactivated deer serum showed greatly decreased activity. Our results suggest that *B. garinii* is sensitive to deer serum, in the presence of antibody and the bactericidal effect is important for preventing Lyme disease in wild sika deer.

**Key words:** *Borrelia garinii*, Complement-mediated killing, Antibody, Wild deer serum

Lyme disease is a complex, multisystemic illness affecting skin, joints, the nervous system and the heart (15). The etiologic agent (4) was first considered to be a single species *Borrelia burgdorferi* (8). On the basis of DNA relatedness, *B. burgdorferi* complex was found to be composed of several genomic species (1). *B. garinii* was isolated from *Ixodes persulcatus* feeding on sika deer in Hokkaido, Japan.

An important aspect of the disease is the ability of the bacterium to survive in the host for months to years after initial onset of disease. Recently, we reported that percentage of seropositive deer was high and seasonal variation of antibody levels was observed in Hokkaido (7). Serum antibodies against *B. burgdorferi* have been detected in the hyperendemic areas of the northeastern United States (3, 13). The role of wild deer in the epidemiology of *B. burgdorferi* complex is not completely understood. Certain investigators contend that white-tailed deer are incompetent as reservoirs for *B. burgdorferi* (16). They suggest that deer may help maintain *B. burgdorferi* in nature but that spirochetes in deer have not been shown conclusively to be infective to ticks. Lane et al (12) suggest that additional studies are needed to determine more precisely the reservoir competence of white-tailed deer for *B. burgdorferi*. Recently, Oliver et al (14) reported that experimentally inoculated deer can serve as a source of *B. burgdorferi* to *Ixodes dammini* larvae and nymphs for at least several weeks.

It has been reported that *B. burgdorferi* is sensitive to human serum in the presence of IgG which mediates bacterial killing through the classical complement pathway (9, 10). To more fully understand the immunological aspects of Lyme disease, we undertook an analysis of possible mechanisms employed by *B. garinii* to survive for a prolonged period of time in an infected natural host and in an infected tick. In this study, we examined the susceptibility of *B. garinii* to the bactericidal activity of wild deer serum.

*Ixodes* ticks were obtained from wild sika deer (*Cervus nippon yessoensis* Heude, 1884) and the midgut was cultured in Barbour-Stoenner-Kelly (BSK) medium (2) previously described (6). Dark-field microscopy was used to screen cultures two times each month. *B. garinii* SIKA2 was one of the isolates from adult female *Ixodes persulcatus*

**Abbreviations:** BSK medium, Barbour-Stoenner-Kelly medium; MEM, Eagle’s minimal essential medium; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; GVB; gelatine veronal-buffered saline.
feeding on sika deer, and was identified by Fukunaga et al (5). The strain was passaged 3 times from the first isolation in medium at 32 °C, and used for experiments.

Blood was obtained from 41 of wild sika deer in east Hokkaido, Japan (43°12′- 43°30′N, 143°24′- 144°12′E), between July and October 1991. Average age was 1.44 ± 1.46 years old (Mean ± S.D.) in 19 females and 1.50 ± 1.90 years old in 22 males, respectively. Age was determined by M. Suzuki and N. Ohtaishi (Department of Oral Anatomy, School of Dentistry, Hokkaido University, Sapporo, Japan), using criteria for sika deer (replacement or cementium annuli of first incisors). Blood samples were allowed to clot for 30 min at room temperature and then for 2 hr at 4 °C. Serum (supernatant) was obtained and stored at 4 °C for 1 to 3 days. The serum samples were transferred from study sites in east Hokkaido to laboratory, and stored at −80 °C until needed. After centrifugation at 2,000 × g, the serum was used. All serum samples were screened for B. garinii-reactive antibodies by enzyme-linked immunosorbent assay (ELISA) previously described (7).

The serum bactericidal assay was done by the method by Kochi et al (9, 10). The bacterial cell concentration was determined in a Petroff-Hausser counting Chamber. The cells were adjusted to 3 × 10⁷/ml in Eagle’s minimal essential medium (MEM) with Hanks salts (pH 7.4; GIBCO). The reaction mixture consisted of bacterial cells (final concentration; 1:10) and deer serum (final concentration; 1:10) in Veronal-buffered saline (GVB, pH 7.4, containing 0.15 mM CaCl₂, 1.0 mM MgCl₂, and 0.1% [w/v] gelatine). Reaction mixtures were incubated at 37 °C for 1 hr with periodic shaking. Afterward, the percent cell survival was determined by removing 10-µl aliquots for examination by dark-field microscopy and by counting triplicate samples. Cells were considered killed when loss of motility (extensive surface blebbing and loss of refractility was associated with loss of motility) was observed. Results were compared with those for control tubes which contained MEM in place of serum.

B. garinii SIKA2 was tested with fresh deer serum for bactericidal action. We divided the samples into 3 groups because the bactericidal activity varied in each serum. In the first group (serum with low killing activity: up to 90% of these cells survived), 5 of 41 sera were included (Table 1). Spirochetes could be detected in the midgut of Ixodes ticks feeding on the deer. In contrast, antibody level was lower than the positive border line (Optimal density: 0.690 at 492 nm). In the second group (1–90% survival of B. garinii), 24 of 41 sera were included. Spirochetes could be detected in the midgut of Ixodes ticks feeding on 3 of the 24 deer. Antibody was positive in 7 deer. In the third group (serum with high killing activity: less than 1% survival of B. garinii), 12 sera were included. No spirochetes could be detected in the midgut of Ixodes ticks feeding on the deer. Eleven of 12 deer showed positive antibody level.

After treatment with fresh deer serum, 93.8% of the spirochetes survived in the serum of deer with spirochete-positive ticks (Table 2). Survival percent decreased when there were no isolation of spirochetes from ticks or no detection of ticks on the deer (P < 0.01). After inactivation of serum, there were no differences between the groups. Recovery of bactericidal action was observed when rabbit fresh serum (complement source) was added to the inactivated deer serum. Rabbit fresh serum (control) alone had no bactericidal effect on the spirochetes (survival percent: 95.7 ± 11.1).

### Table 1. Bactericidal action of deer serum

<table>
<thead>
<tr>
<th>% survival of B. garinii</th>
<th>Positive No. of serum sample/examined(%)</th>
<th>Antibody to B. garinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90</td>
<td>5/41</td>
<td>0/5</td>
</tr>
<tr>
<td>1–90</td>
<td>(12.2)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>&lt;1</td>
<td>(58.5)</td>
<td>(29.2)</td>
</tr>
</tbody>
</table>

* Significantly different from the other groups.

### Table 2. Percent of survival of B. garinii after treatment of fresh deer serum and effect of inactivation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Isolation of spirochetes from feeding-ticks</th>
<th>No isolation of spirochetes from ticks or no detection of ticks on deer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh serum</td>
<td>93.8 ± 19.9</td>
<td>16.0 ± 15.5*</td>
</tr>
<tr>
<td>Inactivated</td>
<td>88.8 ± 12.5</td>
<td>83.6 ± 13.2*</td>
</tr>
<tr>
<td>Inactivated serum + complement</td>
<td>68.2 ± 18.1</td>
<td>2.4 ± 13.2*</td>
</tr>
</tbody>
</table>

* Significant differences were observed.

NS: not significant.
Data for antibody level against B. garinii are shown in Fig. 1. The bactericidal activity of the deer serum were directly related to the antibody level (Spearman’s rank coefficient $r = -0.81$, $P < 0.01$). The serum with a high level of antibody showed a strong bactericidal effect (percent survival of B. garinii was low in the serum).

Our data show that the bactericidal activity of deer serum is related to the level of antibody to B. garinii. Actually, it was difficult to isolate the spirochetes from ticks feeding on deer with high antibody level. Strong bactericidal activity of serum in natural host appear to be an important factor determining the ability of the infecting organisms to survive in the hosts, mammals and ticks.

Telford et al (16) suggested the incompetence of deer as reservoirs of the Lyme disease spirochetes in U.S.A. because spirochetes were detected in about 1% of the nymphs developed to the stage while infection was detected in 23% of field-swept nymphamal ticks. We considered that the percentage was significantly lower than that of field-swept nymphamal ticks. In our study, spirochetes were easily cultivable from the midgut of ticks feeding on antibody-negative deer, whereas they were not cultivable (or cultivable in few samples) from ticks feeding on antibody-positive deer. Recently, it has been reported that experimentally inoculated deer can serve as a source of at least two geographic strains of B. burgdorferi to I. dammini larvae and nymphs for at least several weeks (14). Our results presented here do not settle the controversy as to whether sika deer serve as reservoirs for B. burgdorferi complex. However, the study expects that sika deer from north Japan are susceptible to B. garinii and the spirochetes are maintained in the wild animals without antibody to kill the organisms.

It has been reported that complement-dependent killing of B. burgdorferi requires IgG antibody and is mediated by the classical pathway (9). The mechanism of the antibody action on B. burgdorferi is different from these implicated in antibody action on other Gram-negative bacteria investigated (10). They suggest that the antibody induces a qualitative modification in the manner that C5b-9 interacts with the borreliae. Their current results show that IgG Fab fragments derived from bactericidal antibody are able to mediate killing of B. burgdorferi, thus directly demonstrating that bactericidal effect of anti-B. burgdorferi IgG is independent of the complement-activating properties of the antibody (11). Our results showed that killing occurred only in the presence of specific antibody. The bactericidal systems of deer serum may be similar to that observed in the systems of human serum.

References


