Preliminary Observations on Ultrastructure of Borreliae in Tissues of *Ixodes persulcatus*

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Abstract: We observed Lyme borrelia by electron microscopy in the tissues of the ticks, *Ixodes persulcatus*, which were indicated positive for borreliae by BSK cultures of their internal organs. Borreliae (0.25 μm in diameter) were found only in the lumen of the midgut. They were closely associated with the microvilli on the midgut epithelium but never penetrated into the epithelial cells. Ultrastructural features common to Lyme borreliae, i.e., the three-layered membranes surrounding the cytoplasm and orientation of the flagella insertions, were obviously confirmed. The present results are useful to understand tick tissue-borrelia interface.

Key words: Borrelia, *Ixodes persulcatus*, Ultrastructure

It has been known that Lyme disease is caused by a spirochete, *Borrelia burgdorferi*, transmitted by ticks of the genus *Ixodes* in North America (3). Recent reports on human cases of Lyme disease in Japan, which result from the bite of an ixodid tick, *Ixodes persulcatus* (8, 10), have elevated further scientific interest in tick-borne zoonotic diseases. Under the stimuli of these reports, spirochetes close to *B. burgdorferi* have been frequently isolated from several species of ticks (7, 11-13) and some rodents (11) in Japan. However, ultrastructural studies on Japanese borreliae in tick tissues have not yet been conducted. In the present study, we attempted to observe the ultrastructural features of the borrelia by electron microscopy, taking notice of the tick tissue-borrelia interface.

Unfed adults of *I. persulcatus* (16 females and 11 males) were collected by flagging flannel cloth over vegetation at Mt. Aka-usagi (at 1,000-1,500 meter elevation) in Fukui Prefecture in June 1992. A block of internal organs including midguts, Malpighian tubules, salivary glands, and ovaries or testes was dissected aseptically from each tick and divided into halves. In order to ascertain borrelia-infected ticks for ultrastructural observations, each of the half blocks was inoculated into BSK medium within a culture tube. The tube was incubated at 32 C for six weeks and examined for spirochetes by phase contrast microscopy every two or three days. The spirochetal isolates were identified by an indirect immunofluorescence test (IF) (9) using monoclonal antibodies H9724 (genus-specific for *Borrelia* spp.) and H5332 (specific for the outer-surface proteins of *B. burgdorferi*; the prototype B31). The remaining organ blocks were individually fixed in cold 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 6 hr, and washed thoroughly in buffer. These were postfixed with 1% osmium tetraoxide in buffer for 3 hr, dehydrated with acetone, and embedded in epoxy resin. Thin sections (70 nm) were cut on a microtome (Reichert-Nissei Ultracut N) and doubly stained with uranyl and lead acetate before observation with an electron microscope (Hitachi H-600, 75 kV) (14).

Of 27 adults examined, three (11.1%, two females and one male) were positive for spirochetes on 17 to 30 days after incubating within BSK medium. These isolates reacted with the H9724 monoclonal antibody but did not with H5332 (Table 1). Therefore, it was certain that these isolates belonged to the genus *Borrelia*, while these might not be identical with *B. burgdorferi*. According to Baranton et al (1), it seems likely that our isolates may belong to the genospecies (group VS461) different from *B. burgdorferi* and *Borrelia garinii*.

Ultrastructural observations were made only on the specimens of borrelia-infected ticks indicated by BSK incubation mentioned above. Borreliae were...
found in the midguts of all of three positive ticks, but were not found in any other organs so far as examined. As the number of borreliae found in the midguts was very few, it was considerably difficult to find them in every section. Irregularly coiled borreliae existed in the lumen of the midgut and closely associated with the microvilli on the midgut epithelium but never penetrated into the epithelial cells (Figs. 1 and 2). However, low percentage of ticks with systemic infections of *Borrelia* has been reported in *Ixodes ricinus* (4), *Ixodes pacificus* (5) and *Ixodes dammini (=Ixodes scapularis)* (6). Especially, *Borrelia* infections of oocytes are occasionally found in engorged *I. dammini* females (6). Further observations on tissues from feeding and engorged *I. persulcatus* ticks are needed.

In the cross section, the cytoplasm of the borrelia showed round shape with the diameter of 0.25 μm. Fibrillar strands, presumably representing DNA, were visible in the electron-dense cytoplasm. Three-layered membranes surrounding the cytoplasm were well defined, i.e., cytoplasmic membrane, cell wall, and the outer sheath: they tightly abutted each other. Flagella were inserted parallel with the long axis of the cell between the outer sheath and the cell wall. The flagella varied from eight to ten in number and were situated one-sidedly in the cross section of the borrelia cell (Fig. 1 inset). The number of the flagella in a cross section is said to depend upon the site of the cell because of the overlap of flagella in the middle position (2).

Ultrastructural features of the borrelia observed in the present study corresponded generally to those reported for *B. burgdorferi* in the U.S.A. (2, 3), based on appearance of the three-layered membranes surrounding the cytoplasm and orientation of the flagella insertions. This is the first report of an electron microscopical study on borrelia in tick tissues in Japan. As other tick species are also

<table>
<thead>
<tr>
<th>Tick No.(sex)</th>
<th>Days for borrelia-positive in BSK</th>
<th>Mab-reactivity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(♀)</td>
<td>17</td>
<td>H9724 256 &lt;8</td>
</tr>
<tr>
<td>2(♀)</td>
<td>17</td>
<td>256 &lt;8</td>
</tr>
<tr>
<td>3(♂)</td>
<td>30</td>
<td>256 &lt;8</td>
</tr>
<tr>
<td>4-27(14♀,10♂)</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Titters indicated by reciprocal of dilution of monoclonal antibody (Mab) in IF test.
known to be infected by borreliae (7, 13), further studies on these ticks are extensively required.

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References


