Ultrastructure of Spirochetes Isolated from *Ixodes ricinus* and *Ixodes dammini*

KARI HOVIND-HOUGEN, Ph.D., D.Sc.

State Veterinary Serum Laboratory and Statens Seruminstitut,
Copenhagen, Denmark

Received January 23, 1984

Two strains of *Ixodes* spirochetes, one isolated in the United States (B31) and the other in Sweden (G25), were examined by electron microscopy. Cells of strain G25 were 11–25 μm long with a wavelength of 2.1–2.4 μm and an amplitude of 0.4 μm. Eleven flagella were inserted subterminally at each end of the cell. Cells of strain B31 were similar but had eleven or seven flagella. Cytoplasmic tubules were not seen in cells of either strain. Although not identical, both strains showed ultrastructural details characteristic of the genus *Borrelia.*

Spirochetes can be grouped according to morphological characteristics [3], and the ultrastructure, as seen by electron microscopy, is of value in determining the genus and species of a particular spirochete [3,6]. In the present study two strains of *Ixodes* spirochetes, one isolated in the United States and the other in Sweden, were studied. These preliminary results suggest that the strains are similar and belong to the genus *Borrelia.*

MATERIALS AND METHODS

The two isolates examined were strain G25 Danderyd, which was recovered in Sweden in 1982 from the tick *Ixodes ricinus* by B. Hederstedt, in collaboration with B. Sköldenborg, G. Stiernstedt, B. Svenungsson, and H. Jörbäck [unpublished], and strain B31, isolated in New York state, from specimens of *Ixodes dammini* by Burgdorfer et al. [1]. Both isolates have been linked to Lyme disease serologically.

Both strains were propagated by cultivation at 37°C for five days in a modified Kelly's medium [1]. The cultures were examined immediately after the end of the growth period, and, in some cases, after storage at 4°C for up to five weeks.

All cultures were centrifuged at 1,500 g for 20 minutes and the pellets obtained were resuspended to a suitable density in a few drops of SMC (0.03 percent sucrose in redistilled water supplemented with 0.01 M MgCl₂ and 0.01 M CaCl₂). Specimen grids for electron microscopy were prepared and negatively stained with 1 percent ammonium molybdate by the multiple drop technique [5]. Prior to staining, some cells were treated on the grids for one to two minutes with 2 percent sodium deoxycholate in redistilled water.

Electron microscopy was carried out by the routine methods of the laboratory [5], and the results presented are based on the study of approximately 200 electron micrographs.
RESULTS

Negatively stained cells of strain G25 Danderyd were 11–25 \( \mu \text{m} \) long with a rather regular wavelength of 2.1–2.4 \( \mu \text{m} \) and an amplitude of 0.4 \( \mu \text{m} \) (Fig. 1). The cells had pointed ends and their width increased gradually over about one wavelength to the full width of 0.3 \( \mu \text{m} \).

Eleven flagella were inserted subterminally at each end of the cell (Fig. 2). The insertion points of the flagella were arranged in a row parallel to the long axis of the cell. The flagella wound around the cytoplasmic body and those originating at one end overlapped in the mid region with those originating at the other end. The shafts of the flagella were 17 nm thick. Some cells showed signs suggestive of an extra layer exterior to the outer membrane.

The cells were seen to divide by binary fission. The earliest sign of a division was

**FIG. 1.** A regularly waved cell of strain G25 Danderyd (\( \times \) 14,000). The bar = 100 nm.

**FIG. 2.** An end of a cell of strain G25 Danderyd (\( \times \) 95,000). The flagella (F) are inserted in a row parallel to the long axis of the cell (arrowheads). OM and CM denote outer membrane and cytoplasmic membrane, respectively.

**FIG. 3.** The mid-region of a cell of strain G25 Danderyd (\( \times \) 95,000). A few insertion points (arrows) or new flagella are visible. F denotes flagella.
the presence, in the mid-region of the cell, of points from which short flagella extended (Fig. 3). At a later stage the daughter cells became separated by their cytoplasmic membranes, but were still connected by a mutual outer membrane (Fig. 4). At this stage of the division process, the two new ends appeared truncated (Fig. 4).

Negatively stained cells of strain B31 were 12–17 μm long with a wavelength of 2.8 μm and an amplitude of 0.4 μm. These cells also had pointed ends and reached their full width of 0.3 μm at about one wavelength from the tips of the cells. Eleven or seven flagella were inserted subterminally at each end of the cells (Figs. 5 and 6). Generally, each cell had the same number of flagella at both ends. The flagella were inserted in a row parallel to the long axis of the cell, and those from one end overlapped in the mid-region with those from the other end. Some cells seemed to possess a regularly structured surface layer.

FIG. 4. A dividing cell of strain G25 Danderyd (× 95,000). The daughter cells are separated by their cytoplasmic membranes (CM) but are connected by a mutual outer membrane (OM). F denotes flagella. A few insertion points are seen (arrowheads).

FIGS. 5, 6. Ends of cells of strain B31 with eleven (Fig. 5) and seven (Fig. 6) flagella (F) inserted in a row parallel to the long axis (arrowheads). The outer membrane (OM) and the cytoplasmic membrane (CM) are clearly seen. Fig. 5: × 91,000. Fig. 6: × 95,000.
FIGS. 7, 8. Cell remnants of cells of strain B31 with eleven (Fig. 7) and seven (Fig. 8) flagella ($\times$ 91,000). The cells were treated with sodium deoxycholate. The basal complex of the flagellum consists of a pair of discs (D) connected to the hook (H) situated at the end of the flagellar shaft by a short rod. Flagella partly covered with a sheath were occasionally seen (arrow, Fig. 8).

Flagella and membrane-like debris were the only cell components left on the supporting film of the grid after treatment of B31 cells with sodium deoxycholate (Figs. 7 and 8). The basal complex at the insertion ends of the flagella could be resolved and was seen to consist of a pair of discs connected to the hook of the flagellum by a short rod (Fig. 8). The shafts of the flagella were found to be either 16 nm (Fig. 7) or 21 nm (Fig. 8) thick, and occasionally the thicker flagella seemed to be covered by a sheath (Fig. 8).

After five days of culture, both strains contained cells at different stages of growth. The morphology of these cells was unchanged in cultures held at 4°C for up to five weeks.

DISCUSSION

Although very similar in ultrastructural details, the cells of the two strains are not identical. The length and wavelength of individual cells differ, and cells of strain B31 have either seven or eleven flagella at each end.
ULTRASTRUCTURE OF IXODES SPIROCHETES

TABLE 1
Morphological Characters of Cells of Treponema, and Borrelia and of Spirochetes Isolated from Ixodes ricinus (G25) and I. dammini (B31)

|                  | Length* | Wavelength* | Width* | No. of Flagella | Cytoplasmic Tubules | Division     |
|------------------|---------|-------------|--------|-----------------|--------------------|--------------|
| Treponema        | 4-18    | 0.9-1.8     | 0.1-0.3| 1-8             | +                  | Constriction |
| Borrelia         | 12-23   | 1.7-2.0     | 0.4-0.5| 15-30           | -                  | Septum       |
| G25              | 11-25   | 2.1-2.4     | 0.3    | 11              | -                  | Septum       |
| B31              | 12-17   | 2.8         | 0.3    | 7-11            | -                  | ?            |

*All measurements in μm

Dividing cells were commonly observed in strain G25, and the division took place with obvious septum formation, similar to cells of the genus Borrelia [4]. In strain B31, dividing cells were seen less often. The few dividing cells observed were all in an early stage of division, so, at present, it is uncertain whether cell division in this strain takes place by simple constriction or after formation of true septa. Cytoplasmic tubules [3] were not seen in cells of either strain.

It is important to establish whether the Lyme spirochetes belong in the genus Treponema, Borrelia, or in some other genus. In Tables 1 and 2, it can be seen that cells of strain G25 have morphological similarities to cells of the genus Borrelia. Therefore, on morphological criteria alone, this strain can be regarded as a Borrelia. Yet, it should be noted that the cell-ends are not as sharply pointed and the number of flagella inserted at each end are not as great as for previously reported Borrelia spp. However, only five species [2;6;unpublished] of Borrelia have been examined by electron microscopy of negatively stained material. Thus, the variation in the shape of the ends of the cells and in the number of flagella may be larger than hitherto observed.

It is likely that strain B31 also belongs to the genus Borrelia. However, a variation was noted in wavelength and in the number of flagella inserted at each end, which suggests that this culture consists of two types of spirochetes. The cells of one type are similar to, if not identical with, the cells of strain G25, but the other type have only seven flagella inserted at each end and a slightly larger wavelength. Because some thicker flagella were observed in cultures of B31 cells, it is tempting to believe that these sheathed flagella are derived from cells with the lower number of flagella. This variation in flagella cannot be seen by light microscopy.

TABLE 2
Ultrastructural Characters of Flagella Isolated from Cells of Treponema, and Borrelia and from Spirochetes Isolated from Ixodes ricinus (G25) and I. dammini (B31)

|                  | Basal Complex | Sheath | Width of Shaft* |
|------------------|---------------|--------|-----------------|
|                  |               |        | Sheathed/Naked  |
| Treponema        | Gram-positive type | +      | 18/10           |
| Borrelia         | Gram-positive type | -      | ^/13            |
| G25              | ?             | -      | ^/17            |
| B31              | Gram-positive type | ±      | 21/16           |

*In nm

^Sheath not observed

? Not known
It should be emphasized that this manuscript is a preliminary report about the morphology of Lyme disease spirochetes. Further studies are necessary on the ultrastructure of cloned cells of strain B31. More important, patient isolates need to be examined to determine whether Lyme disease is caused by the G25 type of spirochetes, by the "seven flagella kind" which were seen in cultures of strain B31, or by both.

REFERENCES

1. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP: Lyme disease—a tick-borne spirochetosis? Science 216:1317–1319, 1982
2. Hovind-Hougen K: Electron microscopy of *Borrelia merionesi* and *Borrelia recurrentis*. Acta Path Microbiol Scand B 82:799–809, 1974
3. Hovind-Hougen K: Determination by means of electron microscopy of morphological criteria of value for classification of some spirochetes, in particular treponemes. Acta Path Microbiol Scand B (Suppl 255):1976
4. Hovind-Hougen K: Treponema and Borrelia morphology. In The Biology of Parasitic Spirochetes. Ed by RC Johnson. New York, Academic Press, 1976, pp 7–18
5. Hovind-Hougen K, Birch-Andersen A: Electron microscopy of endoflagella and microtubules in *Treponema Reiter*. Acta Path Microbiol Scand B 79:37–50, 1971
6. Karimi Y, Hovind-Hougen K, Birch-Andersen A, Asmar M: *Borrelia persica* and *B. baltazardi* sp. nov.: Experimental pathogenicity for some animals and comparison of the ultrastructure. Ann Microbiol (Inst Pasteur) 130 B:157–168, 1979