SHORT COMMUNICATION

Pseudospirochaetes in Peritoneal Exudates of Mice Injected with *Bacillus aneurinolyticus*

By HISANORI KONISHI* AND ZENSAKU YOSHII

*Department of Microbiology, Yamaguchi University School of Medicine, 1144 Kogushi, Ube-shi,
Yamaguchi-ken 755, Japan*

(Received 5 July 1985)

Bodies initially thought to be spirochaetes were observed in the ascites of mice injected intraperitoneally with *Bacillus aneurinolyticus* (KA bacillus). Electron microscopy revealed that these bodies were in fact 'pseudospirochaetes' originating from the flagellar bundles of the bacilli.

INTRODUCTION

Bacillus aneurinolyticus Kimura et Aoyama (KA bacillus) is one of the thiamin-decomposing bacilli and has peritrichous flagella (Aoyama, 1952; Gibson & Gordon, 1974). During experiments to estimate the infectivity of KA bacilli by intraperitoneal injection of mice, many spirochaete-like bodies (SLBs) were unexpectedly observed by dark-field microscopy of peritoneal exudates. They were at first thought to be spirochaetes, contaminating or growing in symbiosis with the KA bacilli, although none showed active motility. The electron microscopic study described below showed that the SLBs were in fact 'pseudospirochaetes' composed of flagella detached from the bacilli.

METHODS

Bacillus aneurinolyticus S232, which has been maintained in our laboratory, was grown on nutrient agar plates at 37 °C for 24 h. The culture was suspended in sterile saline solution for injection. Four groups of five mice were injected intraperitoneally with 1.2×10^5 , 1.2×10^6 , 1.2×10^7 and 1.2×10^8 organisms per animal, respectively. One day after injection, peritoneal exudate from each mouse was harvested with a capillary pipette and examined by dark-field microscopy.

The peritoneal exudate in which SLBs were seen was washed once in human serum and three times in distilled water. Part of this material was mounted on grids with a supporting membrane. Some specimens were negatively stained with 2% (w/v) phosphotungstic acid, and others were shadow-cast with chromium in an evaporator before examination with a transmission electron microscope (JEM-100B, JEOLCO, Japan, 80 kV). The other part of the material was fixed with 2% (v/v) glutaraldehyde/1% (w/v) osmium tetroxide, critical-point dried, and coated with gold/palladium in an evaporator. Specimens were also examined with a scanning electron microscope (ISI-DS 130, Akashi, Japan, 25 kV).

The spiral handedness was determined on printed photographs, carefully prepared so as not to make mirror images. Right- (or left-) handedness was defined as rotation clockwise (or counter-clockwise) while moving along the spiral away from the observer (Yoshii, 1978; Carleton *et al.*, 1979).

Abbreviation: SLB, spirochaete-like body.

Fig. 1. Dark-field micrograph of SLBs in the peritoneal exudate of a mouse injected with 1.2×10^8 KA bacilli. A, SLBs; B, KA bacilli. Bar, 10 μ m.

Fig. 2. Scanning electron micrograph of a SLB. Bar, 1 μ m.

Fig. 3. Transmission electron micrograph of chromium-shadowed SLBs. Bar, 1 μ m.

RESULTS AND DISCUSSION

SLBs were observed by dark-field microscopy in the peritoneal exudate from three mice injected with 1.2×10^7 and from all five mice injected with 1.2×10^8 organisms per animal. No SLBs were seen in exudates from mice injected with 1.2×10^5 or 1.2×10^6 organisms per animal. Many SLBs (arrows A in Fig. 1) were seen among the KA bacilli (arrows B). These SLBs were 12–16 μm long and 0.5–1.0 μm wide at the centre, and had tapering ends and five to eight regular waves, and closely resembled certain spirochaetes, such as *Treponema* or *Borrelia*. However, none of them showed active motility.

Scanning electron microscopy revealed that the SLBs were spiral bundles of fibrils, wide in the centre and with tapering ends (Fig. 2). The spiral conformation of all the SLBs was left-handed. No particular features were found on the cell bodies of the KA bacilli except for the loss of flagella. In chromium-shadowed specimens (Fig. 3), SLBs connected with the cell bodies of KA bacilli were occasionally observed and the fibrils composing them were obviously flagella. Moreover, the ultrastructure of each fibril was identical with that of bacterial flagella in specimens negatively stained with phosphotungstate (not shown).

From these results we conclude that the SLBs were not spirochaetes but 'pseudospirochaetes' originating from flagella detached from KA bacilli. It has been reported that when peritrichously flagellated bacteria swim in viscous media containing gum or methylcellulose, the flagella attached to each cell associate into bundles, each with the appearance of a rotating helix (Pijper, 1946, 1947) and that they swim by rotation of the helical bundle of flagella (Berg, 1974; Silverman & Simon, 1974).

The spiral conformation of the SLBs was left-handed, as was found by Macnab & Koshland (1974) and Shimada *et al.* (1975) for flagellar bundles of *Salmonella* strains. This suggests that KA bacilli may also possess flagella with left-handedness and that they swim by rotation of the left-handed helical flagella in a counterclockwise sense, although the helical handedness of individual flagella was not determined in this study.

Although there have been several reports of pseudospirochaetes, most of them (Noguchi, 1926; Rahman & Macis, 1979; Frerichs & Maley, 1980; Sparling & Baseman, 1980; Faine, 1982) describe 'pseudoleptospires' (spiral elements like leptospire), which have been frequently encountered in blood cultures and mistaken for true leptospire. The difficulty in differentiating leptospire from pseudoleptospires has been stressed. However, little attention has been given to other sources of pseudospirochaetes. Noguchi (1926) reported another type of pseudospirochaetes, like *Treponema* or *Spironema* (*Borrelia*), originating from aggregated flagella detached from peritrichously flagellated contaminants during the cultivation of spirochaetes *in vitro* and emphasized the possible occurrence of pseudospirochaetes not only in blood cultures but also in other *in vitro* studies. In the present study, pseudospirochaetes of the same type and origin as described by Noguchi (1926) were observed *in vivo*. Therefore, the possible occurrence of pseudospirochaetes in experiments with flagellated bacteria should be borne in mind for *in vivo* as well as *in vitro* studies.

This investigation was supported by 20th grant from Watanabe Memorial Fund (Ube, Japan).

REFERENCES

- AOYAMA, S. (1952). Studies on the thiamine decomposing bacterium. I. Bacteriological researches of a new thiamine decomposing bacillus, *Bacillus aneurinolyticus* Kimura et Aoyama. *Acta Scholae medicinalis Universitatis in Kioto* **30**, 127–132.
- BERG, H. C. (1974). Dynamic properties of bacterial flagellar motors. *Nature, London* **249**, 77–79.
- CARLETON, O., CHARON, N. W. & O'BRIEN, S. (1979). Helix handedness of *Leptospira interrogans* as determined by scanning electron microscopy. *Journal of Bacteriology* **137**, 1413–1416.
- FAINE, S. (1982). *Guidelines for the Control of Leptospirosis*. WHO Offset Publication no. 67. Geneva: World Health Organization.
- FRERICHS, G. N. & MALEY, A. D. (1980). Pseudoleptospires in blood culture. *Journal of Clinical Pathology* **33**, 905–906.
- GIBSON, T. & GORDON, R. E. (1974). *Bacillus*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 529–550. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- MACNAB, R. & KOSHLAND, D. E., JR (1974). Bacterial motility and chemotaxis: light-induced tumbling response and visualization of individual flagella. *Journal of Molecular Biology* **84**, 399–406.
- NOGUCHI, H. (1926). Abnormal bacterial flagella in cultures. *Journal of the American Medical Association* **86**, 1327–1329.

- PIJPER, A. (1946). Shape and motility of bacteria. *Journal of Pathology and Bacteriology* **58**, 325-342.
- PIJPER, A. (1947). Methylcellulose and bacterial motility. *Journal of Bacteriology* **53**, 257-269.
- RAHMAN, M. & MACIS, F. R. (1979). Pseudo-leptospires in blood culture. *Journal of Clinical Pathology* **32**, 1226-1227.
- SHIMADA, K., KAMIYA, R. & ASAKURA, S. (1975). Left-handed to right-handed helix conversion in *Salmonella* flagella. *Nature, London* **254**, 332-334.
- SILVERMAN, M. & SIMON, M. (1974). Flagellar rotation and the mechanism of bacterial motility. *Nature, London* **249**, 73-74.
- SPARLING, P. E. & BASEMAN, J. B. (1980). The spirochetes. In *Microbiology*, 3rd edn, pp. 751-762. Edited by B. D. Davis, R. Dulbecco, H. N. Eisen & H. S. Ginsberg. Hagerstown: Harper International.
- YOSHII, Z. (1978). Fundamental considerations and experimental conditions in the study of the spiral-direction of helical bacteria. *Bulletin of Yamaguchi Medical School* **25**, 115-127.