Ultrastructure of a mycoplasma recovered from the bone marrow in systemic lupus erythematosus

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In earlier studies, the isolation of mycoplasmas from the bone marrow of patients suffering from systemic lupus erythematosus (SLE) and from synovial fluid in rheumatoid arthritis was reported (Jansson, Vainio, and Tuuri, 1971b; Jansson, Mäkisara, Vainio, Vainio, Snellman, and Tuuri, 1971a). These isolates were shown to have at least partial antigenic identity with *Mycoplasma arthritis*.

In the present investigation, the structure of one of these isolates was studied by thin sectioning and compared with that of *M. arthritis* grown under identical conditions.

Material and methods

Organisms

M. arthritis strain PG 6 was kindly supplied by Dr. R. H. Leach. Strain 338-M was isolated from the bone marrow specimen of a woman with SLE (Jansson and others, 1971b). It did not convert into bacteria when penicillin and thallium acetate were omitted from the culture medium. Electron microscopic studies were performed on its 4th culture passage on cell-free media.

Propagation

The culture medium has already been described (Jansson, 1964). Instead of PPLO broth, brain heart infusion broth was used, and the medium was further enriched with egg yolk, pasteurized at +60° for 50 min., and added in the ratio 0.1 ml. to 10 ml. broth. The strains were grown in a 250-ml. bottle containing 100 ml. enriched mycoplasma broth medium. It was inoculated with 20 ml. of a 5-day culture of *M. arthritis* and strain 338-M respectively, and incubated at +37°C. for 5 days.

Preparation for electron microscopy

To the 5-day culture as well as to the uninoculated culture medium, 100 ml. of 12.5 per cent. glutaraldehyde in a 0.2 M phosphate buffer pH 7.2 were added to a final concentration of 6.25 per cent and kept at +4°C. for 2 hrs. The centrifugation was performed at 14,000 × G for 30 min. The pellet was washed in a buffer containing 0.2 M sucrose in 0.2 M phosphate buffer pH 7.2. Finally 10 ml. of this same buffer was used to store the pellet.

The pellets were post-fixed with 1 per cent. osmium

tetroxide, dehydrated in ethanol series, and embedded in Epon 812 (Luft, 1961). Thin sections were cut with a Porter-Blum MT-1 ultramicrotome, using a diamond knife. The thin sections were post-stained with lead citrate (Reynolds, 1963). Electron micrographs were taken with a Siemens Elmiskop I A electron microscope at original magnifications of 10,000 to 40,000 × and enlarged as desired.

Results

M. arthritis

The specimens contained mostly roundish particles 450 to 650 nm. in diameter, but also elongated, filamentous ones measuring 1 µm. or more in length (Fig. 1, overleaf).

The morphology of the particles varied. They were surrounded by an undulating membrane to which material was often attached, forming a uniform 'outer layer'. At a distance of 150 to 200 Å beneath the unit membrane, there regularly occurred a thin layer of electron dense material which in some instances showed a membrane-like appearance (Fig. 1, inset).

The internal structure of the particles varied considerably. Some showed very sparse electron lucent nucleoid areas. Apart from ribosome-like granules, they contained dense 30 to 50 nm. granules and also fairly homogenous, densely-stained material (Fig. 1), giving the whole particle a very dense appearance. Some showed only loosely packed ribosome-like granules in addition to a well-developed nucleoid area. No elementary bodies could be identified with certainty in the preparations of *M. arthritis*.

Strain 338-M

Specimens of this isolate contained particles which in size and structure closely resembled those of *M. arthritis*, showing mostly a clear double-layered surface structure (Fig. 2A). These specimens also contained in abundance particles which occurred within large aggregates of finely granular material. These particles were mostly surrounded by a single dense layer 70 to 150 nm. thick. A unit membrane

FIG. 1 Thin sectioned *M. arthritidis* particles, showing wide structural variability. At higher magnification (inset), a particle with a typical double-layered surface structure is seen adjacent to a particle surrounded by a single unit membrane. $\times 50,000$; inset $\times 100,000$. Bars = 200 and 100 nm, respectively

structure could very rarely be resolved within this layer (Fig. 2 B, inset). A double-layered surface structure was only occasionally seen in these particles. Many particles showed infoldings of the outer layer, suggestive of different stages of binary fission (Fig. 2 B). No structures resembling elementary bodies could be found within the particles or in the aggregates surrounding them.

The uninfected control preparations were found to contain mostly large aggregates of similar appearance to those in which strain 338-M particles occurred.

Discussion

The present results indicate that strain 338-M, identified as mycoplasma by colony morphology and immunological means (Jansson, Vainio, and Tuuri, 1971), also has the general structural characteristics of mycoplasma as seen in thin sections (Edwards and Fogh, 1960; Freundt, 1960; van Ijtersen and Ruys, 1960; Domermuth, Nielsen, Freundt, and Birch-Andersen, 1964; Hummeler, Armstrong, and Tomasini, 1965; Anderson and Barile, 1966; Anderson and Manaker, 1966; Dienes and Bullivant, 1967; Maniloff, 1969). The strain 338-M particles structurally resemble *M. arthritidis* particles fairly closely,

except for those occurring and apparently also multiplying within the lagre aggregates.

There seems to be general agreement that mycoplasmas are surrounded by a single-unit membrane (for references, see Anderson, 1969). On the other hand, a capsule-like additional layer has been observed in some mycoplasma species (Domermuth and others, 1964) and a flexible double membrane has been described for one mycoplasma strain (Hummeler and others, 1965). Fairly large species variability seems to exist also in the internal structure, especially in the occurrence of different types of cytoplasmic granules, vacuoles, and the formation and frequency of elementary bodies. On the other hand, a clear dependence of mycoplasma ultrastructure on growth conditions, the growth phase,

FIG. 2 Strain 338-M particles occurred both free (A) and aggregate-bound (B). The particles in A show a similar double-layered surface structure to *M. arthritidis*. The aggregate-bound particles (B) often occur in long rows and show infoldings suggestive of binary fission. The surface structure consisting of a single membrane is demonstrated at higher magnification in the inset. A $\times 100,000$; B $\times 50,000$; inset $\times 100,000$. Bars = 100, 200, and 100 nm, respectively

and the preparative processing for electron microscopy has been pointed out (Domermuth and others, 1964; Hummeler, Tomassini, and Hayflick, 1965). It is therefore uncertain whether the structural details observed here, especially the double-layered surface structure, can be regarded as structures specific for these mycoplasma strains.

Summary

The ultrastructure of mycoplasma strain 338-M, originating from the bone marrow of a patient with systemic lupus erythematosus, was studied in thin

sections and compared with *M. arthritis* grown identically in cell-free media. Both strains showed similar particles consistent with the general structure of mycoplasma. The particles were surrounded by a unit membrane under which an additional dense layer was usually discernible. The 338-M strain differed from *M. arthritis* in the tendency of the particles to occur and apparently also multiply within aggregates of growth medium origin.

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