Scanning-beam electron microscopy of mycoplasma isolated from rheumatoid arthritis

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SYNOPSIS Scanning-beam electron microscopic studies on an isolate from definite rheumatoid arthritis are presented.

For five years great efforts were made to develop a modified cell-free medium for fastidious human mycoplasma (Jansson, 1971). Using this method it was possible to isolate mycoplasma from tissue specimens or the joint fluid of several patients with rheumatoid arthritis (Jansson, Mäkisara, Vainio, Snellman, and Tuuri, 1971a; Jansson, Vainio, Snellman, and Tuuri, 1971b). The size of these microorganisms is so small and their morphology so pleomorphic that they are probably overlooked by scientists who are used to studying the large-colony mycoplasma. Scanning-beam electron microscopy studies on one isolate are now presented here.

Material and Methods

ORGANISM
Strain 146-M was recovered from the synovial fluid of a man with definite seropositive rheumatoid arthritis. Figure 1 presents its microscopic appearance after Dienes staining, with a 1,000-fold magnification. Scanning-beam microscopy was performed on this isolate in the 12th culture passage on cell-free media.

PROPAGATION
The culture medium was described earlier (Jansson, 1971). Instead of human serum unheated horse serum was used. The strain was grown in 50 ml centrifuge tubes containing 15 ml of enriched mycoplasma broth medium. The medium in each tube was inoculated with 1 ml of 10-day mycoplasma culture. Cultures were incubated at 37°C for eight hours and two, four, six, and 10 days. They were inoculated at various times and were harvested simultaneously.

SCANNING-BEAM ELECTRON MICROSCOPY
All centrifugation was performed at 4,000 rpm/min for 20 min at 5°C in the CAT 821 head of an International portable refrigerated centrifuge model PR-1. Sedimented mycoplasma were washed three times in Kappa buffer as described by Kammer, Pollack, and Klainer (1970). Three washes were done. Mycoplasma suspended in ice-cold Kappa buffer, lacking 2-mercaptoethanol, were fixed by

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slowly adding 0-25% glutaraldehyde to a final concentration of 25% and gently rotating the suspension for two hours. Fixed cells were washed three times in sterile, distilled water and prepared for scanning-beam electron microscopy according to the method of Klainer and Betsch (1970). Then the specimens were examined in a Cambridge stereo-scan electron microscope (mark II).

Results

Figures 2 and 3 illustrate the appearance in scanning-beam electron microscopy of strain 146-M isolated from definite rheumatoid arthritis. In the eight-hour broth culture spherical cells, some with very thin and short filaments, were seen (Fig. 2). The diameter of the cells was 0-5-1-5 μm. In the two- to four-day cultures large clusters of tightly packed round forms were seen with distinct interplasmic bridges. A few budding cells were also observed. Longer cultivation revealed pearl-band-like arrangements of the cells, some of them showing flattening. In the 10-day broth culture both microcolonies and individual cells appeared. In addition, larger budding forms were noticed with the diameter of 2-5 μm (Fig. 3).

The results indicate that strain 146-M has a real life cycle with a pleomorphism of various developing forms.

Comment

The scanning-beam electron microscopic appearance of strain 146-M isolated from definite rheumatoid arthritis reveals a close resemblance to the morphology and growth cycle of M. pneumoniae as described by Kammer et al (1970). However, long, branching filaments were not seen. This may be attributed to the composition of the culture medium, because otherwise their method for preparation of the specimens was followed. The medium used by us was developed for cultivation of fastidious human mycoplasma. It contained pasteurized egg yolk. When some of our isolates in their first passages were grown in enriched broth without egg yolk they sometimes showed fragile longer forms.

The data just presented add support to the assumption that strain 146-M really is a mycoplasma.

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References