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Isolation of Cell Wall Deficient
Mycobacterium tuberculosis from
a Case of Chronic Arthritis

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Synovial fluid from a case of pyarthrosis was
cultured for fungi, mycobacteria, aerobes and
anaerobes with negative results. A Cell Wall
Deficient (CWD) form was isolated in numerous
cultures which showed the physico-chemical
reactions characteristic of wall deficient M. tu­
berculosis.

Bacteria with atypical morphology are
being encountered with increasing frequen­
cy in chronic joint disease. It has been
suggested that disease previously consid­
ered hypersensitivity in joint spaces may
actually be chronic infection with wall defi­
cient organisms.

Wall deficient or cell wall deficient is
currently a useful term to describe L-forms
and other “atypical” organisms with varied
amounts of wall and diverse ability to revert
to the classical stage. Thus cell wall defi­
cient microbes include L-forms, L-phase,
transitionals, spheroplasts and protoplasts.

In the described case the organisms did
not revert to the classical stage although the
morphology became almost typical. It
seems unnecessary to further define the
variant other than wall deficient.

Case History

The patient is a 34-year-old male who was
admitted to the hospital with pyarthrosis of the
left knee four weeks after synovectomy and re­
moval of osteochondritic lesions of the medial
femoral condyle. The specimen taken consisted
of two pieces of soft pink tissue. Sections of the
specimen showed a markedly inflamed mass of
fibrous tissue, numerous histiocytes, as well as
lymphocytes and polymorphonuclear neu­
trphiles. Numerous foreign body giant cells
were also seen.

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LeBar, Mattman and Ross

Figure 1
Colonies which appeared on media adjoining a disc containing a relatively low concentration of isoniazid.

Materials and Methods

Cultures. Routine cultures were made on blood agar (BBL) and incubated in 10% CO₂ and anaerobically by the gas pack method (BBL). An additional culture for anaerobes utilized chopped meat and the V.P.I. method. Cultures for mycobacteria were made on Lowenstein-Gruft slants (Difco) and incubated in 10% CO₂. Attempts to culture fungi employed Sabhi agar (Difco), supplemented with 7% human blood. The Sabhi tubes were duplicates, incubated at 37 C and room temperature.

Cultures for Mycoplasma, were made following Chanock’s method, on Mycoplasma agar (Difco) supplemented with 20% agamma horse serum (BBL) and 10% yeast extract (Fleishman’s 20-40). The microscopic colonies which appeared on this medium after 72 hours were not typical of Mycoplasma, and were subcultured by transferring a block of the agar to Kirchner’s medium* containing 50 units/ml of streptomycin. After incubation at 37 C for one week, the growth in Kirchner’s medium was used as inoculum for surface streaking and pour plates of the following medium: Trypticase soy agar (BBL) with the addition of 1% calcium gluconate and 10% human plasma. Three series of plates were made containing respectively 50, 500, and 1000 micrograms/ml of isoniazid. After incubation in 2% CO₂ for 72 hours, the resultant growth was stained by a variety of methods.

Staining. The surface growth was spread over slides and the following stains which are specific for acid fast species applied: Kinyoun’s stain,* the triple stain,* and the acid-fast acridine orange stain.* These three stains were employed in the intensified method which reveals the acid-fastness of wall deficient mycobacteria.* The Auramine-rhodamine stain as modified by Truant* was also used.

As a simple stain to reveal morphology for microphotography, 1.0% aqueous basic fuchsin was applied to surface and sub-surface growth on the INH plates. Blocks of agar were placed on a cover slip and flooded with the fuchsin.

Immunofluorescence. Fluorescent antibody studies employed H37Ra antiserum (Difco) diluted 1:6 and commercial fluorescein-labelled antirabbit globulin diluted 1:30. Controls consisted of smears of wall deficient variants which had the characteristics of Neisseria, and a second culture which reacted with antiserum to Pseudomonas aeruginosa.

Results

No classical growth was ever obtained during six weeks of incubation of the media for mycobacteria and fungi. No growth appeared on blood agar or in cooked meat during incubation.

Four colony types were noted in the subcultures from the Mycoplasma agar. Some colonies had an irregular outline with moderately swollen inclusions. These are shown in Figure 1. Colonies of the second type contained large vesicles and were seen in relation to a high concentration of INH. Types three and four included cocci, short rods, and a syncytial type of growth. Not every colony was acid-fast, but examples of every type of colony were found to be acid-fast by Kinyoun’s, the triple stain, and the acridine-
Cellwall Deficient Bacteria in Arthritis is from a Case of Chronic Arthritis

orange acid-fast stain. The triple stain had the advantage of giving the most marked contrast between the red acid-fast colonies and the de-colorized background. Growth which stained green in the triple stain was not counted as acid-fast since Candida and a few other genera of organisms in addition to mycobacteria appear green by this method.

Many microcolonies had small beaded rods at their periphery, with morphology typical for M. tuberculosis. Nevertheless, complete reversion never occurred, ie, transfers to Lowenstein's slants never revealed macroscopic colonies.

A 4+ fluorescence was obtained with the labelled antibody to H37Ra. The most marked reactions were with variants on plates near 1000 μgm discs of isoniazid.

Discussion

The variants retained the stain when subjected to auramine-rhodamine. Had the stain not been retained this would have ruled out identification as mycobacteria. However, for CWD forms this is not proof of mycobacteria, as wall deficient variants of some other species may also retain the fluorescent stain.12,13

The methods used in this investigation are the ones which are useful to characterize the wall deficient variants in bacteremia of untreated pulmonary tuberculosis. The growth in a 48-hour blood culture is usually acid fast by Kinyoun's and by the triple stain when these are intensified.14 The growth is also acid-fast by the acid-fast acridine orange stain when intensified, but this stain requires special care in the decolorizing process, and is more difficult to use than the other two.

The growth was also classified as mycobacterial by its stimulation by INH. This amounts to antibiotic-dependence, since relatively large microscopic colonies appear on medium which gives almost no growth when the antibiotic is absent. Antibiotic-dependence in wall deficient fungal variants has been noted by Meinecke14 and in bacterial variants by Barth.15 In the case of bacteria it may be that growth occurs without the antibiotic, but in units too fine to be resolved with the compound microscope. The effect, at any rate, is apparent growth only in association with certain concentrations of certain antibiotics. The use of this principle in identification of cell wall deficient variants of genera other than mycobacteria remains to be expanded. It is obviously a paradox that an antimicrobial agent which is inhibitive in vivo is stimulating in vitro. A possibility is that the antibiotic changes the microbe to a state more susceptible to antibody or to phagocytosis.

Thus, the wall deficient organism in this case was identified as M. tuberculosis by its reaction with specific antiserum, its acid-fastness, its reaction to INH, and the morphology of semi-reverted units. This laboratory diagnosis fits with the patient's history of chronically infected tissue containing giant cells, and failure of a good response to antibiotics directed against pyogenic organisms.

In the past, CWD variants of M. tuberculosis, including filtrable forms, have been revealed in a great variety of tissues of infected individuals by successive passage in guinea pigs. The subject has recently been reviewed.10 However, to our knowledge, this is the first demonstration of wall deficient mycobacteria in synovial fluid by employing immunological and staining reactions, and specific reaction with an antimicrobial agent.

References


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