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Structure of *Mycobacterium leprae*

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Introduction: why study structure?

Information about the structure of *M. leprae* may be obtained by microscopy (especially electron miscroscopy) and other physical techniques. There is active development of such techniques, but at present the information they can give about structures as complex as a bacterium is limited. To obtain a detailed knowledge of the molecular composition and construction of *M. leprae* there is still a need for «dissection» and analysis of bacterial cells, using substantial amounts of material. Since this is difficult (and expensive) to obtain, it is perhaps worthwile to define what one hopes to learn from studying *M. leprae* in this way.

The natural environment of the leprosy bacillus is a eucaryotic cell, usually human but (as is now becoming clear) occasionally that of an armadillo (1) or a monkey (2). For the majority of *M. leprae* the cell is a macrophage, though from the point of view of the host the fact that other sorts of cells become infected may be important in the disease. From this one may infer two functions of bacterial structure: to ensure phagocytosis (in the absence of specific antibodies) by suitable cells, and to ensure survival in the hostile environment inside them. *M. leprae* must also multiply inside its host cell, and it seems possible that some features of its structure will be modified from those of a «typical» mycobacterium to allow most efficient use of the limited range of nutrients available. There is at least some evidence that the bacteria modify the behaviour of the cell, particularly its interactions with other cells involved in cell-mediated immunity, to their advantage (3). The nature and site of the bacterial products having this effect are of interest.

A second important aspect of the structure of *M. leprae* is involved with attempts to interfere with the pathology of leprosy, by protective or therapeutic vaccination or immunomodulation. One would like to know what bacterial structures are recognized by T cells and which of them are needed to stimulate protective immunity, what (if any) processing by antigen-presenting cells occurs and what is the nature of the processed products.

Third, a knowledge of special structures in *M. leprae* may suggest new types of anti-leprosy drugs which can interfere with their synthesis. Further, an understanding of the structures of the surfaces of the bacteria would possibly help in designing particular derivatives of drugs best able to penetrate the bacterial cells.

Finally, at least one convenient way of identifying *M. leprae* and distinguishing it from other acid-fast bacteria is to detect specific bacterial components. Such identification is useful in diagnosis and also in confirming that cultivated bacteria are, indeed, *M. leprae*.

The capsule

That *M. leprae* has a capsule was originally suggested by Hanks (4), on the basis of light-microscopy of isolated bacteria. The electron-transparent zone (5) discovered by electron microscopists was suitably situated for a capsule, but there was debate about whether it was a bacterial or host-cell structure (6) and since it was entirely featureless in sections little further

information could be obtained. Techniques for seeing the surfaces of bacteria – negative staining and latterly freeze-fracture and freeze-etching – have demonstrated that *M. leprae* occurs surrounded by a vesicular or foamy structure not seen around other species of intracellular mycobacteria (7). The same material seems to form the «foam» of the Virchow cell, originally observed by light microscopy (8).

The key to the identification of the capsular material came from the observation the homogenates of infected tissue contained large amounts of bacterial lipid that could be separated mechanically from the bacteria (9). That the bacterial capsule was composed of these lipids seemed overwhelmingly likely. (It should be noted, however, that the lipids have not yet been formally identified *in situ*.) Two main bacterial lipids are involved, both having structures unlikely to be susceptible to breakdown by host-cell enzymes. Phthiocerol dimycocerosate (10, 11) is a highly apolar substance which seems likely to have a purely passive protective function; the phenolic glycolipid (12) (PGL-I being the main member of a group of related lipids) may play a more active role. Mice may be able to mount a weak cell-mediated immune response to the phenolic glycolipid, (13) but as far as is known at present, neither of these substances stimulates delayed hypersensitivity in man, though the phenolic glycolipid is serologically active (14, 15) and, since it does not occur in any other recognized species of mycobacterium, is of importance for identification of *M. leprae*. It may also have a cell-modifying function, since it is found to suppress the proliferation of lymphocytes caused by concanavalin A (16).

Tissues and cells infected with *M. leprae* also contain unusually high levels of cholesterol esters (also author's unpublished results) (17), so the host may contribute towards the lipid capsule. It would be interesting to know how nutrients gain access to the bacterial cell through the thick, hydrophobic capsule.

The wall

The *M. leprae* cell is surrounded by a wall, an organelle possessed by almost all eubacteria which defines the shape of the cell and protects it from the environment. As seen in freeze-etched preparations, the wall is smooth and featureless save for a few radial bands apparently each consisting a pair of narrow raised lines (18). These bands are not unique to *M. leprae*; (19) they may be scars left by cell division. In thin sections in the electron microscope the wall consists of two major layers, (20) of which the inner takes up the heavy-metal stains used to visualize objects in the electron miscroscope and the outer is transparent to electrons. A variable amount of electron-dense material may occur on the outside of the transparent layer, possibly residues from the capsule. (21)

The chemical nature of the wall has been worked out in detail for mycobacteria other than *M. leprae,* (22, 23) and in outline for *M. leprae* itself. (24) The chemical identification of the layers observed in thin sections has been worked out for *Mycobacterium lepraemurium* (25). The inner layer is peptidoglycan, a polymer common to most bacteria, consisting of chains of repeated disaccharide units cross-linked with short peptides. The structure in *M. leprae* is identical with that in other mycobacteria except in one respect: L-alanine in the cross-linking peptide is replaced by glycine (P. Draper, A. Darbre & O. Kandler, unpublished results). This variant of peptidoglycan is rare, and otherwise occurs in members of the family *Actinoplanaceae,* which are filamentous soil bacteria related to the mycobacteria. (26)

The electron transparent layer of the wall consists of lipopolysaccharide – a branched chain arabinogalactan esterified with long-chain mycolic acids. The mycolic acids of *M. leprae* differ in detail from those of other mycobacteria (27) but they are of a type confined to mycobacteria, and this is one strong piece of evidence for the conventional classification of the organisms. Too little of the arabinogalactan from *M. leprae* has been available to check

whether the precise structure is the same as that of other mycobacteria, but the materials are clearly similar. Thus the wall of *M. leprae* is very like that of other mycobacteria and a similar puzzle applies to all the species: how is the lipopolysaccharide, of which over half is lipid, arranged so that nutrients can reach the bacterial cell? One possible arrangement has been suggested in which the lipids form a hydrophobic surface layer (28). This agrees well with the physical properties of many mycobacteria (including *M. leprae*) but would make access by hydrophilic substances difficult.

Some observations based on negative staining also suggest that the arrangement of the lipopolysaccharide is more complex. One model suggested the presence of several layers, each containing rope-like or linear structures embedded in a matrix (29, 30). It is likely that these observations were confused by the presence of capsular materials as well as the true wall, but the structures seem to exist in the innermost part of the wall as well further out. Also, isolated and purified walls are ornamented by «paired fibrous structures», (31) not usually seen on intact bacteria; capsular material is unlikely to be present in purified walls. A knowledge of the true structure of the wall of *M. leprae* (and other pathogenic mycobacteria) is an important objective. It is also important to know whether there exist any wall-associated proteins, such as occur in many other types of bacteria. There is no definitive evidence for these so far in any mycobacterium.

The membrane

The wall is a passive molecular sieve, with as yet unknown effects on the diffusion of molecules of various sizes and polarities. The underlying membrane controls transport in and out of the bacterial cell. Its properties have been well studied in various bacteria, including *Mycobacterium phlei* (32), but almost nothing is known of the membrane of the pathogens. There is some evidence that the membrane of *M. leprae* differs from the rest, since the two electron-dense leaflets are of the same rather than of different thicknesses, at least in most individual cells. (33) In other species loss of viability is accompanied by loss of membrane asymmetry, (34) but some *M. leprae* with symmetrical membranes appear morphologically normal and probably viable.

The membrane presumably contains, as well as the usual polar lipids (which are similar in *M. leprae* to other mycobacteria), (35) proteins controlling active or passive transport and probably enzymes for synthesizing the wall and perhaps the capsular lipids. Many of these components are excellent candidates for «selective toxicity» for new drugs. They may also be accessible to antibodies or cells of the host – may indeed be «surface protein antigens».

The contents of the cell

The contents of *M. leprae* fall more into the realm of metabolism than structure. Unlike eucaryotic cells, most bacteria have little in the way of organelles except ribosomes and storage granules, both of which have (unsurprisingly) been found in *M. leprae* (21, 36). Complex mesosome-like bodies have repeatedly been described in thin sections (37). Originally credited with several functions, mesosomes in most bacteria are now often dismissed as artifacts of degenerating cells. It is not clear whether this applies to mycobacterial cells. In the case of *M. leprae* it is exceedingly hard to determine the functional state of individual cells and their intracellular location makes rapid fixation difficult. It seems likely that the reality of the mesosome-like structures will have to wait for the cultivation of the organism to be determined.

The DNA of the bacterial cell is not demarcated from the cytoplasm and its arrangement is not yet certain. However, the chemical properties of the molecule are of interest. The DNA of *M. leprae* is about the same size as that of *M. tuberculosis,* but its base composition is somewhat different from that of all the other mycobacteria (55 % guanine plus cytosine

rather than 65-70 %). (38) (39) It is possible that this is an adaptation to intracellular existence. It does not necessarily indicate that *M. leprae* is not closely related to other mycobacteria, nor that the proteins it contains are greatly different (the immunological evidence shows they are, on the contrary, very similar).

Conclusion

The two current requirements – for new anti-leprosy drugs and for a vaccine – should be more easily be fulfilled if some of the remaining puzzles about the structure of *M. leprae,* and particularly its capsule, wall and membrane, are solved. Some of the solutions will be reached by inference from knowledge of other mycobacteria, but the unique nature of this pathogen makes it likely that important structural features are also unique, and that the organism itself, as well as models, must be studied.

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