WORKSHOP 13: MICROBIOLOGY

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Sources. The nine-banded armadillo still provides bulk quantities of *Mycobacterium leprae* of acceptable microbiological quality, so long as protocols for controlling contamination are followed. Smaller quantities of *M. leprae* of high quality (high viability, low chance of contamination) are available from homozygous athymic nude mice. In some regions of the world, adequate supplies for research are not available.

Purification. The "IMMLEP 1/79" procedure yields suspensions comparable in viability with unpurified suspensions. Other satisfactory methods involving density-gradient centrifugation exist; one appears to fractionate *M. leprae* cells according to density (possibly related to intactness). A small-scale method for purifying *M. leprae* from human biopsies needs to be developed.

Structure and composition. Evidence is emerging for the presence in *M. leprae* of a 30-kDa protein like that secreted by *M. bovis*. Capsular material can be observed electron microscopically and may form the electron-transparent zone of intracellular mycobacteria. "Buried" proteins linked to the peptidoglycan of *M. leprae* apparently exist. Most components of the envelope (some unique to *M. leprae*) have been identified and monoclonal antibodies, potentially useful in studying ultrastructure, raised to most. *M. leprae* (like *M. tuberculosis*) inhibits phagosome-lysosome fusion in phagocytic cells.

Molecular biology. Five immunodominant antigens were cloned and two have been sequenced. As SDS-denatured proteins they are recognized by antibodies and by some T cells. A repeated sequence of DNA has been discovered in *M. leprae*; probes for this may be useful for detection and identification. Expression of *M. leprae* genes has been obtained in *Escherichia coli* and *Streptomyces*. There are encouraging prospects of cloning *M. leprae* genes in cultivable mycobacteria. An immunomodulatory fusion protein has been reported. Genes coding for identifiable enzymes have been expressed: citrate synthetase and biotinylated proteins (probably acyl-CoA carboxylases, involved in lipid synthesis). Analysis of the sequence of 16S rRNA placed *M. leprae* phylogenetically in the group of slow-growing mycobacteria; analysis of the sequence of the ribosomal RNA gene gave preliminary indications of strain differences in *M. leprae*.

Biochemistry. *M. leprae* apparently depends on the host for purines but is able to synthesize its own pyrimidines. It can synthesize fatty acids *de novo* and can use the glycoxylate shunt. However, phosphotransacetylase seems deficient; acetate is incorporated into lipids only in intracellular *M. leprae*. Palmitate is incorporated into phenolic glycolipid-I (PGL-I) and is also readily oxidized. Iron-chelating molecules have not been detected but those from *M. neoaurum* can be utilized. Cytochrome B1, but no others, has been identified. Catalase levels are very low but can be detected by immunoprecipitation methods.

Viability testing and drug screens. Systems were described for assessing viability and drug susceptibility *in vitro*. Different systems might be optimal for each purpose. It was noted that compounds have different relative activities in different systems. It is

essential to use good quality bacteria for drug screening. Assays for viability varied greatly in sensitivity. Palmitate catabolism appeared to be a particularly promising assay for drug screening; assays based on synthesis of macromolecules will probably be useful. No results of comparisons by double-blind trial of existing *in vitro* drug screens are available.

Cultivation. Two new tissue culture systems seem promising; *M. leprae* multiplied in mouse fibroblasts (but lost infectivity) and also in Schwann cell cultures. Axenic multiplication has been described: in the presence of "adjuvant" mycobacteria; in conditioned medium; in a microaerophilic medium (coccoid forms); in normal media. Identification criteria (see below) should be applied to all these cultures with precaution to ensure that the initial inoculum is diluted out.

Identification. Use of several or all of the following are recommended for identification of cultures claimed to be *M. leprae*: mycolic acids (types by high-resolution chromatography after alkaline methanolysis; species by GC-MS); PGL-I (using monoclonal antibodies); G+C content of DNA (56%); DNA hybridization. Restriction fragment analysis will be valuable when fully developed. Reaction with specific monoclonal antibodies to the "famous five" antigens is suggested with the caveat that three of them appear to be stress proteins and may not be expressed in cultured *M. leprae*. Immunodiffusion analysis can rule out known, cultivable mycobacteria. Growth in normal mouse foot pads and nerve involvement are characteristic (though not technically taxonomic tests).