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CELL WALLS OF *MYCOBACTERIUM LEPRAE*

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Bacterial cell walls are important to bacteria for maintaining their shape and supporting them against osmotic stresses. They also form the primary defence of the bacterial cell against the environment. The chemical and physical structure of walls are also important to taxonomists; several of the schemes for the immunological typing of bacterial strains depend on the chemistry of the walls.

The nine-banded armadillo experimentally infected with *M.leprae* provides a supply of these bacteria on an unprecedented scale, and the preparation in our Laboratory of a "soluble" skin-test antigen from purified bacteria also provides bacterial cell walls as a side-product. I have studied these and find that they show several chemical similarities and at least one important difference from the structure of chemically well-studied mycobacterial walls.

Suspensions of bacteria were prepared from livers and spleens of infected armadillos (supplied by Drs W.F. Kirchheimer and G.P. Walsh under the IMMLEP Programme) by homogenisation followed by differential centrifugation and treatment with Triton X100, using the system that I developed for *M.lepraemurium* (1). Residual host components were removed by digestion with collagenase and then Pronase (proteinase from *Streptomyces griseus*), both at 100 μg/ml for 24 hr (or more if necessary) at 37°. The suspensions still contained a small amount of coloured material, which could not be separated from the bacteria on sucrose density gradients. This was removed by suspending the bacteria in an aqueous two-phase system containing polyethylene glycol 6000 and dextran T500 (5% & 7%, respectively, in 0.01M-phosphate, pH 6.9, and 0.01M-NaCl; 2). About 99% of bacteria were found in the upper (PEG-rich) phase while the coloured material was attached to the interface between the two phases. I could see no non-bacterial material in negatively stained suspensions, purified by this method, in

the electron microscope.

The suspensions were broken ultrasonically (20 min at 0°, 6 μm peak-to-peak movement on a 15 sq.mm probe at 20kHz; this treatment gives virtually total breakage of the cells), and centrifuged at 34 900 x g. for 30 min. The sediment was re-suspended and treated with RNAase plus DNAase, followed by trypsin. The walls were banded on a sucrose density gradient (15-60% w/v) at 27 500 x g. for 2 hr, then treated with 1% sodium dodecylsulphate and washed with 0.05% Tween 80, 1M-NaCl, more Tween 80 and finally with water and freeze dried.

"Free" lipids were removed with chloroform-methanol (1:1, by vol) extraction for 4 days, and "bound" lipids with 0.5% KOH in methanol for 4 days at 37°, followed by extraction with chloroform-methanol. The lipid-free walls were dried, then resuspended ultrasonically in water and analysed as follows: total neutral sugars by the phenol-H_2SO_4 method (3); individual sugars by GLC of trimethylsilyl ethers of methyl glycosides (carried out by Mr R. Faulkes); amino acids after hydrolysis in 4M-HCl at 105° for 16 hr followed by automatic analysis (carried out by Miss S. Lathwell). Bound lipids were studied by thin-layer chromatography on silica-gel.

Typically 25 mg (dry weight) of bacteria, obtained from about 13 g of armadillo liver, yielded 4 mg of purified walls and 1 mg of lipid-free walls. Negatively stained, the purified walls appeared in the electron microscope as featureless polygonal fragments about 0.2 μm across.

The bound lipids had the chromatographic properties of mycolic acids, and two components could be separated. The pattern obtained on chromatograms differed from that shown by M.tuberculosis, M.microti, M.lepraemurium and M.vaccae.

The walls contained about 60% of neutral sugars, consisting mainly of arabinose and galactose (molar ratio 3:1) with smaller amounts of glucose and a sugar tentatively identified as ribose. Amino acids and amino sugars were found in molar ratios shown in the Table, in one sample of

walls the molar ratio galactose:diaminopimelic acid was 2.5:1.

TABLE

SAMPLE	AMINOACIDS & HEXOSAMINES					
	DAP	ALA	GLU	GLY	GLCN	MUR
A6W	1.0	0.68	0.88	1.04	0.74	0.48
A7W	1.0	0.79	1.06	1.37	0.65	0.51

MOLAR RATIOS (DIAMINOPIMELIC ACID=1) OF AMINOACIDS AND HEXOSAMINES IN PURIFIED WALLS OF MYCOBACTERIUM LEPRAE

(HEXOSAMINES UNCORRECTED FOR LOSSES DURING HYDROLYSIS)

M.leprae occurs in small numbers of infected armadillo liver compared with M.lepraemurium in infected mouse liver, and it has been necessary to add several steps to our purification method developed for the latter. My confidence that the final suspension of bacterial walls is not contaminated either with bacterial membrane or with residual host material is based on the clean electron microscopic appearance of the bacteria and walls and the absence of common "protein" aminoacids in the walls.

M.leprae appears from these analyses to belong to the actinomycetales, as it contains mycolic acid, arabinogalactan and peptidoglycan in its walls. At least one of the mycolic acids has the chromatographic properties of a mycobacterial mycolic acid, though this must be confirmed by mass spectroscopy. On the other hand the peptidoglycan appears to contain substantial quantities of glycine. The possibility that the glycine derives from contaminant collagen is small; none was seen in the suspensions, and no proline was found in hydrolysates.

The simultaneous occurrence of glycine and diaminopimelic acid in bacteria is rare (4), and it is absent in the myco-

bacteria that have been analysed (5). It is known that bacteria starved of alanine and/or grown in excess of glycine may substitute glycine for alanine in the peptidoglycan (6). M.leprae may well be in a glycine-rich environment in the armadillo, but the possibility of obtaining sufficient bacteria grown in different animal species or on media seems remote at present, so the hypothesis that this composition of the peptidoglycan is phenotypic cannot be tested.

SUMMARY

Walls were prepared from M.leprae isolated from livers and spleens of infected armadillos. These contained mycolic acids and arabinogalactan, but the composition of the peptidoglycan differed from that of the other mycobacteria that have been studied, since it contained substantial amounts of glycine. The relationship of M.leprae to the other mycobacteria remains obscure.

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