IMMUNOLOGICAL DIFFERENTIATION BETWEEN CELL WALLS OF STREPTOMYCIN-SENSITIVE AND STREPTOMYCIN-DEPENDENT BACTERIA*

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Presently available evidence indicates that streptomycin exerts its effect on sensitive bacteria by reacting with ribosomes in such a way as to interfere with or alter the synthesis of proteins (Cox, et al, 1964, Pestka, et al, 1965 and others). Altered enzyme proteins produced by cells in the presence of streptomycin would be expected to result in the synthesis of cellular components, some of which would differ from those existing in cells not exposed to streptomycin. In particular, strains of bacteria which have mutated to become dependent upon the presence of streptomycin would be expected to possess different components than their parent streptomycin-sensitive cells.

We have examined the composition of purified cell walls of streptomycin sensitive and streptomycin dependent strains of several genera and species of bacteria, and have reported that striking quantitative and qualitative differences exist between the two kinds (Lawrence and Scruggs, 1966a,b).

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This communication presents evidence that the cell walls of streptomycin dependent bacteria of several kinds possess a common antigenic determinant which is different from that possessed by the parent streptomycin sensitive strains.

MATERIALS AND METHODS. Streptomycin dependent and resistant mutants of Bacillus megaterium, B. cereus T, Staphylococcus aureus 209 P, Sarcina lutea and Escherichia coli were isolated as colonies which developed on Trypticase Soy agar (BBL) containing streptomycin (1 mg/ml). Cells were harvested from Trypticase Soy broth after 16 hours growth at 37°C on a rotary shaker. The medium for the dependent strains contained streptomycin (1 mg/ml); resistant strains were grown both in the presence (R+) and absence (R-) of streptomycin. The same purified wall preparations as previously described (Lawrence and Scruggs, 1966a) were used. Walls of the other species were obtained in a similar manner. In addition, intact cells and aliquots of the crude cell walls, that is, before treatment with trypsin, were collected.

Rabbits were immunized with crude and purified walls of B. megaterium, and the agglutinating activity of the immune serum was checked with the homologous antigens, as well as with the intact cells and the cell walls of the other species. For immunization, the crude and the purified cell walls of streptomycin-sensitive B. megaterium (Meg S) and of the dependent strain (Meg D) were diluted in sterile saline to a concentration of 1 mg/ml dry weight. The antigens were mixed

with equal volumes of complete Freund's adjuvant, kindly supplied by Dr. Jack Emerson. After a pre-injection bleeding, 2 rabbits each were immunized with the 4 antigens; on day 1 with 0.5 ml intravenously (with the adjuvant omitted), with 1.0 ml intraperitoneally and 1.0 ml subcutaneously. The intraperitoneal and subcutaneous injections were repeated on days 12, 19, and 34. Blood was then collected by cardiac puncture and the serum was stored at -20°C.

The sera were tested for agglutinating activity. For use as test antigens, the cell wall preparations were treated for 3-6 minutes in a 10 KC sonic oscillator, with the samples maintained at temperature <10°C. This period of oscillation sufficed to prevent spontaneous agglutination and settling of the particulate antigens. The re-oscillated cell wall preparations and the intact cells were then diluted with 0.08% NaCl to a concentration which gave an OD_{540} of 0.25-0.28 in a 1 cm. cuvette.

Tube agglutinations were carried out with equal volumes of cell wall preparations or intact cells and dilutions of serum. The tubes were incubated at 56°C for an hour and then at 5°C for 4-5 days. The prolonged holding at 5°C resulted in visible agglutination at higher serum dilutions than did 24 hrs. at 5°C. Continuation of the low temperature incubation beyond 5 days showed no further change.

RESULTS AND DISCUSSION. Pre-immune sera tested against each antigen gave agglutination titers not greater than 1:4.

Agglutination tests with preparations of the same bacterial species (B. megaterium) as was used for immunization (Table 1) demonstrate that streptomycin-dependent cells and cell walls can be distinguished from streptomycin-sensitive preparations by their reaction with the antisera. A common antigen was present in all cases, as would be expected from the common mucopeptide components of bacterial cell walls; however, distinctive antigenic determinants were also present in the two kinds of cells. Walls of streptomycin-resistant B. megaterium cells (R+ and R-) differed from the S and D preparations in that they could not easily be distinguished one from the other

Table 1

Agglutination titers of pooled anti-megaterium sera against fractions of streptomycin-sensitive and streptomycin-dependent B. megaterium.

Immune Serum	Test Antigens							
	Pure cell walls		Crude cell walls		Whole cells		Pure cell walls	
	S	D	S	D	S	D	R(-)	R(+)
against Meg S crude walls	64	8	256	64	256	<4	*	*
against Meg D crude walls	8	32	8	64	8	64	*	*
against Meg S pure walls	128	32	256	16	64	8	16	16
against Meg D pure walls	64	256	16	64	16	256	4	16

*Not tested.
S = fraction of streptomycin-sensitive strain.
D = fraction of streptomycin-dependent strain.

with antisera prepared against the S and D strains. This was also the case with cell walls of R- and R+ strains of *B. cereus T*; however, R- and R+ walls of *E. coli* could be so distinguished, the walls of R- *E. coli* agglutinating with a higher dilution of antiserum against Meg S walls and the walls of R+ *E. coli* agglutinating with a higher dilution of Meg D antiserum (Table 2).

Table 2

Agglutination titers of pooled anti-megaterium sera against fractions of streptomycin-sensitive and streptomycin-dependent strains of other species and genera.

Imm. Serum	Test antigens									
	B.cereus T pure walls†		S. aureus pure walls		S. aureus whole cells		S. lutea pure cells		E. coli pure walls	
	S	D	S	D	S	D	S	D	R(-)	R (+)
Meg S crude	x	x	x	x	32	<4	x	x	x	x
Meg D crude	x	x	x	x	8	128	x	x	x	x
Meg S pure	64	8	32	4	<4	<4	16	<4	32	4
Meg D pure	4	16	8	16	<4	256	8	64	16	256

x Not tested
S = fraction of streptomycin-sensitive strain.
D = fraction of streptomycin-dependent strain.
† = In addition to the pure walls of S & D strains of *B. cereus T*, the pure walls of R(-) & R(+) strains of this organism were tested against antisera to Meg S and Meg D pure walls. In each of these latter two cases, the titer was 1:8.

That this antigenic specificity crosses species and generic lines can be seen in Table 2. Here also common antigens were present, as has been found by several workers using cell wall preparations of various bacteria (Abdulla and Schwab, 1965; Wiseman, 1963; Cummins, 1962).

In the light of the previously reported similarities in mucopeptide composition of walls of streptomycin-dependent bacteria, and their differences from the walls of sensitive strains, it seems likely that the specificities reported here may be attributable to changes in cell wall composition or structure brought about ultimately by the action of streptomycin on the protein-synthesizing system.

References

Abdulla, E. M. and J. H. Schwab, Proc. Soc. Exptl. Biol. Med. 118, 359 (1965).

Cox, F. C., J. R. White, and J. G. Flaks, Proc. Natl. Acad. Sci. U. S., 51, 703 (1964).

Cummins, C. S., J. Gen. Microbiol. 28, 35, (1962).

Lawrence, N. L. and M. E. Scruggs, J. Bacteriol., 91, 1378 (1966a).

Lawrence, N. L. and M. E. Scruggs, Bacteriol. Proc., (1966b).

Pestka, M., R. Marshall and M. Nirenberg, Proc. Natl. Acad. Sci. U. S., 53, 639 (1965).

Wiseman, D., J. Pharm. Pharmacol. 15 Suppl., 182, (1963).