Polyarthritis Induced in the Rat with Cell Walls from Several Bacteria and Two *Streptomyces* Species[1] (37421)

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Water-in-oil (w/o) emulsion containing killed mycobacteria not only increases the production of circulating antibodies to an incorporated antigen but also enhances a delayed hypersensitivity skin response to that antigen. Such an emulsion is almost universally used for immunological investigation and is called Freund's complete adjuvant (FCA) (1). FCA also produces adjuvant arthritis (AA) in the rat (2). It is known that *Nocardia* (3) and *Corynebacterium rubrum* (4) can replace mycobacteria for AA induction. In addition, previous workers have shown that the cell walls of *Mycobacterium, Corynebacterium,* and *Nocardia* have a common structure comprised of a mycolic acid–arabinogalactan–mucopeptide complex (5–8). It is also known that the cell wall materials of these 3 bacterial species are able to produce AA in the rat (9–11). The present paper concerns trial induction of AA by cell wall preparations from various other gram-positive bacteria using a sensitive experimental system comprised of the intra lymph node injection route (12, 13) and the highly susceptible Lewis strain rat.

Material and Methods. Female Lewis inbred rats (Microbiological Associates, Bethesda, Maryland), 10 wk old and weighing 150–160 g, were used throughout this study. The cell wall preparations from various bacteria were processed according to the method described by Iwata *et al.* (14). We have confined our attention entirely to the cell walls of gram-positive species—*Mycobacterium bovis,* BCG (Takeo strain), *Corynebacterium diphtheriae* (P.W.8 strain), *Bacillus megaterium* (KM strain), group A *Streptococcus pyogenes,* type 12 (S.F.42 strain), *Staphylococcus aureus* (Copenhagen strain), *Lactobacillus plantarum* (ATCC8014 strain) and *Micrococcus lysodeikticus* (NC TC-2665 strain). Cell walls from *Streptomyces lavendulae* (IAM0023) and *Streptomyces fradiae* (IAM0093) were obtained through the courtesy of Dr. Teruya Nakamura (Takarashuzo Central Lab, Japan). Injections were made into the inguinal lymph nodes according to Newbould's technique (13). Inocula for lymph nodes totalled 0.01 ml w/o emulsion containing 0.1 mg of most of the cell walls. In the case of BCG and *C. diphtheriae* cell walls, graduated doses (0.004, 0.02, 0.1 mg/rat) were used. Most cell wall preparations were triturated and suspended in mineral oil containing 5% Arlacel A (we have evidence that prolonged exposure of these suspensions at 60° for up to 96 hr produces better results) followed by addition of an equal volume of phosphate-buffered saline pH 7.2 (PBS) to make the w/o emulsion. Since lyophilized *B. megaterium* cell walls were difficult to incorporate into mineral oil, this cell wall preparation was first suspended in PBS and then mixed with mineral oil + Arlacel A. Animals were observed daily for 4 wk after injection. Observation and scoring of AA were done as reported previously (15). The maximal score was 20 with the lymph node injection method, since the 4 paws and tail were each graded from 1 to 4.

Results and Discussion. Cell walls from *M. bovis* BCG, *C. diphtheriae, Streptomyces lavendulae, Streptomyces fradiae* and *L.*

[1] This work was supported by U.S. Public Health Service Grant GM15759 and the Kroc Foundation for Advancement of Medical Science.

TABLE I. Polyarthritis Induction in the Lewis Rat by Injections of Various Cell Walls from Gram-Positive Bacteria into Lymph Nodes in Freund's Incomplete Adjuvant.

Cell walls[a]	Dose	Arthritis incidence[b]
BCG	0.1	7/7
	0.004	7/7
Corynebacterium diphtheriae	0.1	9/11
	0.02	10/10
	0.004	11/12
Streptomyces lavendulae	0.1	7/7
Streptomyces fradiae	0.1	6/7
Staphylococcus aureus	0.1	1/6
Bacillus megaterium	0.1	0/6
Streptococcus pyogenes	0.1	0/11
Micrococcus lysodeiktieus	0.1	0/7
Lactobacillus plantarum	0.1	3/5
No cell walls (w/o emulsion only)	—	0/7

[a] 0.01 ml of w/o emulsion containing each cell wall material was injected into both inguinal lymph nodes.

[b] Arthritis incidence is given by the number of diseased rats divided by the number of rats injected.

plantarum were all able to produce AA, including ancillary lesions (nodules of the ears and nose), whereas the cell walls from *B. megaterium, Str. pyogenes,* and *Micrococcus lysodeikticus* were unable to do so (Table I). The cell walls of BCG and *C. diphtheriae,* in graduated doses of 0.004, 0.02, and 0.1 mg/rat, produced extremely severe AA with high incidence at all three levels (Table II). The time of onset of AA ranged from Days 7 to 12 and the disease usually reached its peak 2–3 wk after injection. Even though the symptoms in both groups of *Streptomyces* (0.1 mg/rat) remained mild to moderately severe, features such as day of onset and development of AA were identical to those in the rats injected with *Mycobacterium* or its components (2, 15, 16) and with *C. rubrum* (4). As seen in Fig. 1, initial changes (on Day 13) may consist of a mild diffuse pink swelling of the ankle, and small paw joints in all 4 paws. Nodular lesions were also observed on the tail, ears, and nose.

Bonhomme (9) reported that *M. tuberculosis* cell walls of both human and non-human origin were capable of producing AA. Azuma (11) also showed that BCG cell walls were strongly arthritogenic, whereas *N. asteroides* cell walls produce mild AA with low incidence and *C. diphtheriae* cell walls produce a 45% incidence of secondary swelling only in the injected paw of Sprague–Dawley rats. Paronetto succeeded in producing AA with *C. rubrum* (4) and showed that arthritogenic activity is localized in the cell walls of that organism (10). Our finding concerning the arthritogenicity of cell walls from BCG and *C. diphtheriae* proved that these cell walls are efficient arthritogens in the rat and that as minute a dose as 4 μg/rat of these cell walls can produce typical AA in high incidence. All clinical features were identical to those produced by *Mycobacterium* bacilli and wax D. We believe that the experimental system used in this study is extremely sensitive and the lymph node method is superior to the conventional intracutaneous or footpad injection method (12). Our successful induction of AA with other cell wall materials like *Streptomyces, S. aureus,* and *L. plantarum* was probably due at least in part, to the use of this sensitive system. Even though cell walls from group A beta-hemolytic streptococci failed to produce AA in this study, a water-soluble, high molecular weight component of this organism (HMWC) proved to be strongly arthritogenic in the rat and these results will be reported on soon (17). We are pursuing further studies along this line with HMWC preparations from different bacteria. It is very probable that the use of active HMWC from cell walls may unmask latent arthritogenic capabilities of various other bacteria.

The ability of *Streptomyces* to produce AA probably can be explained by its taxonomical relationship to Mycobacteriaceae (*Mycobacterium, Corynebacterium* and *Nocardia*). Cummins and Harris (18) reported that *Streptomyces* cell walls containe alanine, glutamic acid, glycine and LL-diaminopimelic acid. The cell walls of *Streptomyces* are distinguished from those of the 3 families of Myobacteriaceae by the fact that they

TABLE II. Comparison of Arthritogenicity of Several Cell Wall Preparations from Various Bacteria in Terms of Onset Day and Severity of Arthritis.

Cell wall material	Dose (mg/rat)	No. of rats	Arthritis	
			Mean onset day[a]	Severity (score/20)
BCG	0.1	7	7.4	12 16 18 18 19 20 20
	0.004	7	9.0	14 18 18 19 19 19 20
Corynebacterium	0.1	11	12.4	2 5 6 7 8 8 11 13 14
diphtheriae	0.02	10	10.4	1 5 6 6 7 8 8 12 12 13
	0.004	12	10.8	3 4 4 4 5 5 6 6 6 7 10
Streptomyces lavendulae	0.1	7	11.3	4 4 5 5 6 7 11
Streptomyces fradiae	0.1	7	11.6	1 1 2 2 3 3
Staphylococcus aureus	0.1	6	15.0	1
Lactobacillus plantarum	0.1	5	16.0	3 3 9

[a] Mean onset day calculated from arthritic rats only.

lack a characteristic sugar such as arabinose and galactose, and also because the LL-isomer is present instead of DL-diaminopimelic acid which is always found in Mycobacteriaceae. Although *S. aureus* cell wall-induced AA remained extremely mild and transient with low incidence (1/6), we have very recently produced severe AA with the same dose of HMWC from *S. aureus* cell walls (17).

As one of the animal models for human rheumatoid arthritis, polyarthritis in the rat which has to date been produced mainly with *Mycobacterium* should be reevaluated in light of the present observations that a wide variety of bacteria are

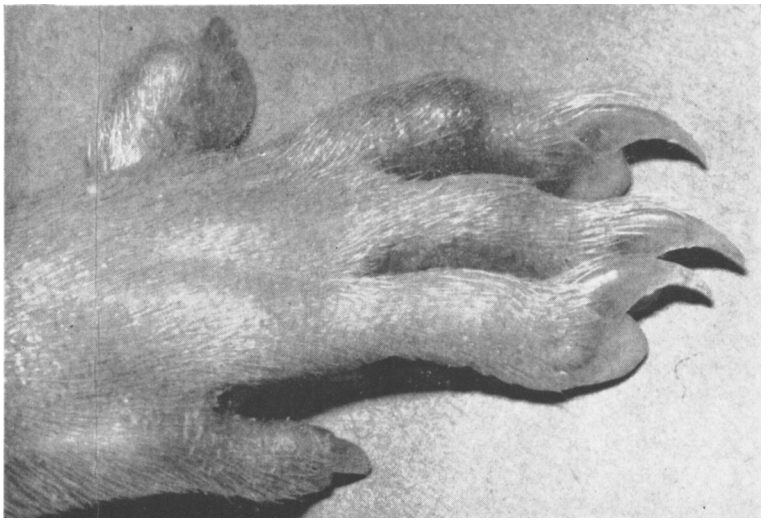

FIG. 1. Polyarthritis of the hind paw produced by injection with *Streptomyces lavendulae* (Day 13).

able to induce this rat disease. Common denominators, either chemical or immunological or both, are being sought in order to further explain the pathogenesis of this interesting disease model.

Summary. Various cell wall preparations from 9 different gram-positive strains were tested for AA induction in the Lewis rat by the lymph node injection method. A striking arthritogenic capability of the cell walls of *M. bovis*, BCG, and *C. diphtheriae* was noted to be essentially equivalent to that of *M. tuberculosis*. The cell walls from *Streptomyces fradiae* and *lavendulae*, *L. plantarum*, and *S. aureus* were less potent, and several other bacterial strains were not arthritogenic at all. However, when high molecular weight water soluble fractions were isolated from some of these strains, they too were often found to be arthritogenic, when administered in water-in-oil emulsions.

We thank Barbara Vande Sande, Robin Yeaton, and Shigeki Nagao for skilled technical assistance.

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Received Mar. 26, 1973. P.S.E.B.M., 1973, Vol. 143.