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Measurement of Streptococcal Cell Wall in Tissues of Rats Resistant or Susceptible to Cell Wall-Induced Chronic Erosive Arthritis

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The quantity of streptococcal cell wall localized in the joints of rats of strains which are either susceptible (Sprague-Dawley, LEW/N, M520/N) or resistant (Buffalo, WKY/N, F344/N) to cell wall-induced chronic erosive arthritis was measured after intraperitoneal injection of group A streptococcal cell wall fragments. Susceptibility or resistance was not associated with a difference in the amount of cell wall localized in limbs or other tissues. It is concluded that although localization of cell wall in joint tissue is essential for development of arthritis, the relative resistance of certain rat strains reflects genetic regulation of inflammatory response rather than a quantitative difference in localization of cell wall in joints.

Systemic injection of rats with an aqueous suspension of cell wall fragments derived from group A streptococci and other bacterial species induces an acute inflammatory arthritis which recedes and is then followed by a waxing and waning course that ultimately progresses to destructive erosive synovitis (2, 3, 6, 8). Outbred Sprague-Dawley and certain inbred strains, including LEW/N and M520/N, are highly susceptible, whereas other strains including Buffalo, WKY/N, and F344/N are relatively resistant to the destructive erosive disease (1, 6–8). Analysis of the genetic control has shown that susceptibility is polygenic. The present study was designed to determine if susceptibility or resistance to chronic erosive arthritis is related to the quantity of cell wall that localizes in joint tissue.

Female rats were obtained from the following sources: inbred Buffalo, Simonsen Laboratories, Bilroy, Calif.; outbred Sprague-Dawley, Zivic-Miller, Allison Park, Pa.; LEW/N, M520/N, WKY/N, and F344/N, Small Animal Section, National Institutes of Health, Bethesda, Md. They weighed 100 to 120 g at the time of injection.

Purified cell walls were isolated from group A streptococci as previously described (5). Briefly, streptococci were grown in Todd-Hewitt broth (BBL Microbiology Systems Cockeysville, Md.), harvested and washed in phosphate-buffered saline (pH 7.4), and disrupted in a Braun MSK shaker (Bronwill Scientific Inc., Rochester, N.Y.). Intact cells were removed by repeated centrifugation at 2,000 × g for 30 min, and the cell walls were collected by pelleting at 10,000 × g for 30 min. After repeated washing, treatment with proteases and RNase, and extraction with methylchloroform, the purified cell walls were washed with water, dialyzed against water, and lyophilized. In the experiment described in Table 1, the cell walls were extracted with 4% sodium dodecyl sulfate instead of methylchloroform (8). Cell wall fragments were prepared for injection by sonication of cell wall suspended in phosphate-buffered saline (20 mg/ml) for 70 min in a Branson sonifier (Branson Sonic Power Co., Danbury, Conn.). The sonicated cell wall was filtered through a Millipore 0.45-μm-pore-size filter before injection. The scoring method for clinical evaluation of arthritis has been described previously (2).

At intervals after intraperitoneal (i.p.) injection of cell wall fragments animals were killed by ether or CO_2, and the tissues were collected and extracted as previously described (4). Equal numbers of control rats were injected with phosphate-buffered saline, and their tissues were processed in the same manner. The amount of cell wall was measured by an enzyme-linked immunosorbent assay modification of the solid-phase radioimmunoassay described by Eisenberg et al. (4). Extracts of tissues were added to 96-well microtiter plates previously coated with rabbit anti-group A polysaccharide which had been affinity purified on an *N*-acetylglucosamine column. Biotinylated affinity-purified anti-group A polysaccharide antibody was then added followed by avidin alkaline phosphatase. The color reaction was developed with *p*-nitrophenylphosphate substrate and read in an automated microELISA reader (Dynatech Laboratories, Inc., Alexandria, Va.).

In the experiment described in Table 1, inbred rats representing susceptible (LEW/N and M520/N) or relatively resistant (WKY/N and F344/N) strains were injected i.p. with cell wall fragments at a dose of 20 μg of rhamnose per g of body weight. Four days after injection the limbs, livers, and spleens were collected for measurement of cell wall by immunoassay.

TABLE 1. Quantitative comparison of group A streptococcal cell wall in tissues of rat strains susceptible or resistant to cell wall-induced arthritis at 4 days after i.p. injection of purified cell wall fragments

Rat strain	μg of cell wall/g of tissue[a]			
	Hind limb	Front limb	Liver	Spleen
Susceptible				
LEW/N	0.54 ± 0.34	1.39 ± 0.25	643 ± 667	209 ± 102
M520/N	0.19 ± 0.05	0.55 ± 0.30	84 ± 9.8	1,264 ± 1,637
Resistant				
WKY/N	0.73 ± 0.71	0.85 ± 0.46	152 ± 115	529 ± 346
F344/N	1.11 ± 1.74	1.37 ± 0.29	111 ± 27	488 ± 120

[a] Mean ± standard deviation; three rats per group

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TABLE 2. Comparison of severity and incidence of arthritis and quantity of streptococcal cell wall in tissues of Buffalo and Sprague-Dawley rats[a]

Tissue	Day after cell wall injection	Buffalo rats			Sprague-Dawley rats		
		Joint score	No. positive/total	μg of cell wall/g of tissue	Joint score	No. positive/total	μg of cell wall/g of tissue
Hind limb	3	0.45 ± 0.6	3/6	0.35 ± 0.2	2.5 ± 1.9	5/6	0.5 ± 0.5
	30	1.5 ± 1.9	1/4	0.7 ± 0.5	3.4 ± 0.6	6/6	0.3 ± 0.1
Front limb	3	0	0/6	0.3 ± 0.06	1.75 ± 0.2	5/6	0.7 ± 0.7
	30	0.6 ± 1.25	2/4	0.8 ± 0.4	2.6 ± 1.4	6/6	0.7 ± 0.6
Liver	3			166 ± 44			158 ± 79
	30			70 ± 15			45 ± 28
Spleen	3			596 ± 181			225 ± 157
	30			121 ± 90			171 ± 105

[a] The right hind limb and right forelimb were collected 3 or 30 days after i.p. injection of cell wall fragments. The joint score was recorded just before sacrifice. Maximum score for one limb is 4.0. Values are mean ± one standard deviation.

In another experiment, relatively resistant Buffalo rats and susceptible Sprague-Dawley rats were injected i.p. with group A streptococcal cell wall fragments at a dose of 40 μg of rhamnose per g, and tissues were collected at 3 and 30 days for quantitation of cell wall. Table 2 compares the joint scores and quantity of cell wall localized in these extremities and other tissues. Despite the marked difference in severity of joint inflammation in both experiments, there was no consistent or significant difference between rat strains in the amount of cell wall in the limbs or other tissues.

Previous reports have shown that the severity of arthritis in susceptible rat strains is correlated with the amount of cell wall localized in the limb (4). It was hypothesized, therefore, that resistant rat strains developed less severe arthritis because less cell wall was transported into their joint tissue. The present study demonstrates that this hypothesis is incorrect and confirms other investigations which used ^{14}C-labeled *Lactobacillus casei* cell walls (6). Although localization of cell wall in the joint is essential for the development of arthritis, the distinction between susceptibility and resistance depends upon factors other than quantity of cell wall distributed to joint tissue.

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