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Arthropathic Properties Related to the Molecular Weight of Peptidoglycan-Polysaccharide Polymers of Streptococcal Cell Walls

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The covalently bound polymers of peptidoglycan and group-specific polysaccharide (PG-APS) were isolated from the cell walls of group A streptococci. Arthritis was induced in rats with a single intraperitoneal injection of an aqueous suspension of PG-APS fragments derived by sonication. The joint lesions induced with this polydisperse suspension followed a bimodal pattern consisting of an acute phase, which reached a peak 5 days after injection and then receded, followed by a chronic, remittent, erosive arthritis lasting several months. The relative severities of the acute and chronic phases could be manipulated by selection of the size of PG-APS fragments. The fragments of PG-APS obtained by sonic treatment were resolved on the basis of size into three major populations by sucrose gradient or differential centrifugation. Based upon light scattering and gel filtration, the average molecular weight of the largest family of fragments was estimated to be about 500×10^6, the intermediate fragments were 50×10^6 daltons, and the predominant size in the smallest population was 5.3×10^6 daltons. The larger fragments induced negligible acute inflammation, but chronic disease became apparent 5 to 9 weeks after injection. The smallest fragments induced the most severe acute inflammation, with relatively little late, chronic joint disease. The particles of intermediate size induced moderate acute inflammation and the most severe chronic, erosive joint lesions. A single injection of fragments of the isolated peptidoglycan moiety of the PG-APS induced only a moderate acute inflammation of joints, with no apparent capacity to maintain the injury and induce chronic disease.

Earlier reports have described the histological, radiological, and immunological features of the chronic, erosive synovitis induced in rats or guinea pigs with cell wall fragments isolated from group A streptococci (4–7). This experimental arthritis is produced with a single intraperitoneal (i.p.) injection of an aqueous suspension of cell wall fragments and has a bimodal pattern in the rat. The first phase is an acute inflammation of joints, which reaches a peak at 3 to 5 days after injection and then recedes. This is followed by a chronic, remittent phase, which first appears at about 3 weeks and is associated with destruction of cartilage. The disease progresses over a period of several months to ankylosis of the joints.

The purified cell wall preparation consists of covalently bound polymers of peptidoglycan (PG) and group-specific polysaccharide (APS). One of our objectives is to define the minimal structural unit of the PG-APS polymer which is required to induce the experimental disease. Previous studies have shown that intact, heat-killed group A streptococcal cells or large fragments of cell wall obtained from mechanically disrupted cells are relatively ineffective (6, 15). That is, intervals of 5 to 20 weeks may elapse before any joint inflammation is apparent, and the incidence of disease is low. More active preparations are obtained by further fragmentation of the cell wall by limited enzymatic digestion with muralytic enzymes or by mechanical degradation by sonication (6, 18). This report relates the arthropathogenic activity of PG-APS to the particle size of the fragments obtained by sonic degradation.

MATERIALS AND METHODS

Bacterial cell culture. Group A, type 3, strain D-58 streptococci were grown in 15-liter batches of Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) in a Virtis fermentor (The VirTis Co.,

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Gardiner, N.Y.) with continuous stirring at 37°C, and the pH was maintained at 7.00 to 7.03 by the automatic addition of 5 N NaOH. The 15 liters of broth was inoculated with 500 ml of an 18-h culture. The initial pH was 7.6, and when this reached 7.0, additional glucose was added continuously in proportion to the NaOH consumed. The final concentration of additional glucose added was 3.0%. The growth was monitored by the periodic measurement of optical density, and by this criterion, the early stationary phase was reached at 7 h. At this time, the cells were collected in a Pellicon filtration unit (Millipore Corp., Bedford, Mass.). The concentrated cells were washed in the filtration unit with 8 liters of sterile phosphate-buffered saline (PBS), pH 7.2. The cells were concentrated to 1 liter and collected by centrifugation at 10,000 × *g* for 30 min.

Cell wall preparations. The washed cells were suspended in approximately 100 ml of PBS (pH 7.2) so that a 1/100 dilution gave a reading of 350 in a Klett-Summerson colorimeter with a green (no. 54) filter. Thirty milliliters of the cell suspension plus 1 drop of tributyl phosphate were added to 30 g of no. 11 glass beads, and the cells were disrupted in a Braun MSK cell homogenizer (Bronwill Scientific Inc., Rochester, N.Y.). Shaking for 3 to 4 min with flowing CO_2 as a cooling agent was sufficient to disrupt over 95% of the cells. Intact cells were removed by centrifugation at 2,000 × *g* for 30 min, and the cell walls were collected by centrifugation at 10,000 × *g* for 30 min (8,500 rpm, Sorvall GSA rotor). The pellet was resuspended in PBS (pH 7.2) and washed three times in PBS. The pellet was thoroughly dispersed at each washing by sonication for 15 s with a Branson model 350 Sonifier (Branson Sonic Power Co., Danbury, Conn.). The cell walls were further purified by treatment with RNase (0.025 mg/mg of cell wall) for 4 h at 37°C with constant shaking. They were washed once with PBS and treated with trypsin (0.025 mg/mg) for 4 h at 37°C, washed again, suspended in 0.1 M phosphate buffer (pH 7.0) containing 0.001 M cysteine and 0.001 M EDTA, and treated with papain (0.020 mg/mg) for 4 h at 37°C. The cell walls were washed twice more in PBS, once in water, resuspended in water, dialyzed against water, and lyophilized. The dried walls were next suspended in a 2:1 dilution of chloroform-methanol (40 mg/ml) and extracted at room temperature for 2 h by stirring. This extraction was repeated two more times. The cell walls were washed once with water, dialyzed against water, and lyophilized. All enzyme preparations were filtered through a Millipore 0.45-μm filter before use. After each sedimentation, the resuspended pellet was dispersed by sonication for 15 s to ensure thorough washing. The cell wall preparations were analyzed for sugars and amino sugars by gas-liquid chromatography and for amino acids by amino acid analyzer as described previously (8). By these analyses the final product was composed of components characteristic of the PG-APS. The cell wall preparations treated only with RNase and trypsin still contained some glucose, which suggested contamination with lipoteichoic acid or glycosyl glycerides of the cytoplasmic membrane. Treatment with papain and extraction with chloroform-methanol removed all detectable glucose.

Preparation of fragments of purified cell wall (PG-APS) by sonication. The lyophilized purified cell walls were resuspended in PBS, pH 7.2 (20 mg/ml), and subjected to sonic vibration for 70 min in a Branson Sonifier by using a 3/4-in. (ca 1.9-cm) probe in a sealed 40-ml stainless-steel cup which was cooled with a water jacket with flowing water at 4°C. The Sonifier was stopped every 20 min for a 5-min period to allow additional cooling. As the final step in the standard preparation of PG-APS fragments for animal injection, the sonicated suspension was filtered through a Millipore 0.45-μm filter. This step removed any intact bacteria and, most importantly, provided a criterion for the maximum size of PG-APS fragments injected. Between 10 and 20% of the PG-APS was removed by filtration.

Resolution of fragments of PG-APS by sucrose gradient centrifugation. After sonication, the undegraded, large pieces of PG-APS were removed by centrifugation at 10,000 × *g* for 30 min in a Sorvall SS-34 rotor. This sedimentation removed 6 to 7% of the PG-APS. Three milliliters of the supernatant (55 mg) was placed on 32 ml of a 10 to 60% gradient of sucrose in PBS, pH 7.2, and centrifuged in a TV-850 vertical rotor (Sorvall) at 50,000 rpm (200,000 × *g*) at 4°C for periods of 0.5, 1, 2, and 5 h. Fractions were collected by pumping 70% sucrose into the bottom of the tube, and the eluate was monitored by optical density at 254 nm. Since there is negligible absorbance of this wavelength by cell wall components, the optical density readings reflect turbidity and not relative concentration. The pooled fractions were dialyzed against several changes of water at 4°C until the dialysate contained no sucrose and lyophilized. Sixty-five percent of the sample was recovered in the three major fractions. The specific gravity of the sucrose gradients was measured with a Zeiss refractometer.

Separation of fragments of PG-APS by differential centrifugation. After sonication for 70 min, the suspension was centrifuged at 1,000 × *g* for 30 min in a Sorvall SS-34 rotor to remove the largest particles (in experiment 3 the suspension was centrifuged at 3,000 × *g*). The supernatant was centrifuged at 10,000 × *g* for 30 min (SS-34 rotor). The pellet from 10,000 × *g* was resuspended in PBS, pH 7.2, and labeled 10p30. The supernatant from 10,000 × *g* was centrifuged at 100,000 × *g* for 60 min in a T-865 rotor in a Sorvall OTD-2 ultracentrifuge. This supernatant from 100,000 × *g* was centrifuged again at 100,000 × *g*, and the final supernatant was labeled 100s60. The pellets from 100,000 × *g* were resuspended in PBS, pH 7.2, for a 30-s sonication and labeled 100p60. Before analyses, this suspension was centrifuged at 10,000 × *g* to remove large aggregates. On the basis of rhamnose determinations, 22% of the recovered fragments were in the 10p fraction, 53% were in the 100p fraction, and 25% were in the 100s fraction. Before being injected into animals, each preparation was sonicated for 2 min to disperse the fragments.

PG. PG was isolated from purified cell walls of strain D-58 group A *Streptococcus* by five successive extractions with formamide at 170°C for 1 h. After the final extraction, the insoluble residue was washed once in water, dialyzed against water for 48 h, and lyophilized. The details of this procedure were described by Krause and McCarty (12). This preparation contained 0.5% rhamnose. To prepare for injection, 100 mg was suspended in 20 ml of PBS and subjected to sonication for 2 min in a Branson sonicator with a 20-ml chamber at a power setting of 8. This yielded a

stable suspension with an optical density at 550 nm of 0.315. The 2-min sonication was based upon previous experience with the biological properties of PG (1, 10).

Another preparation was derived in the same way from cell walls of strain K43 group A variant *Streptococcus*. By gas-liquid chromatography analysis this preparation had a composition of 0.9% rhamnose, 13.3% muramic acid, and 14.5% glucosamine. This preparation, suspended in PBS as described above, was sonicated with the Branson sonicator at a power setting of 5. Samples were obtained before sonication and at intervals of 18, 30, 60, and 150 s of sonication. Each of these samples was injected i.p. into groups of six rats in doses of 3.0, 1.0, and 0.3 mg per 100-g rat to test for arthropathic activity.

Physical and chemical measurements of PG-APS fragments. The procedure and equipment used for light scattering measurements and calculations of molecular weight are described by Carr et al. (3). The refractive index increment (dn/dc) was determined for each fraction by the method of Carr et al (3): 100s = 0.139, 100p = 0.160, and 10p = 0.167. Reducing sugars were determined by the method of Thompson and Shockman (20), and free amino groups were measured by dinitrophenylation as described by Ghuysen et al. (9).

Gel filtration of PG-APS fragments. Bio-Gel A-150 and A-50 agarose columns were prepared according to the manufacturer's specifications (Bio-Rad Laboratories, Richmond, Calif.). Gels were prepared with 0.1 M phosphate buffer, pH 7.2, or 0.1 M ammonium bicarbonate, pH 7.8. Filtration was done at 4°C at a flow rate of 10 ml/h in columns (100 by 1.5 cm) with a void volume of 51 ml. One milliliter of sample (10.0 mg) was added to the column, and fractions were collected in 2.0-ml volumes in an LKB fraction collector.

Scoring of joint disease. The scoring of severity of joint lesions by clinical assessment and by radiological methods has been described (2, 4).

Animals. Outbred female Sprague-Dawley rats weighing approximately 100 g were obtained from Zivic-Miller, Allison Park, Pa.

RESULTS

Particle size of PG-APS fragments obtained by sonication. The decrease in the average molecular weight of the PG-APS polymers with increasing time of sonication was measured by light scattering (Fig. 1). Under the conditions of sonication employed, the molecular weight after 70 min was approximately 500 × 10^6. This represents the average size of the largest particles in this heterogeneous mixture, since smaller particles contribute relatively little to this measurement.

The change in particle size with sonication is a reflection of the breaking of bonds in the cross-linking peptide and in the glycan backbone of the PG. These changes, measured by the increase in free amino groups and reducing sugars (Fig. 2), reached a plateau at about 70 min of sonication under the conditions employed.

Resolution of PG-APS fragments. The heterogeneity of the PG-APS fragments obtained by sonication was analyzed by sucrose gradient centrifugation. Instead of a continuous spectrum of particle sizes, the sonicated PG-APS resolved into four populations (Fig. 3). Each peak moved through the gradient between 0.5 and 5 h, which indicates that the separation was by velocity sedimentation rather than by equilibrium.

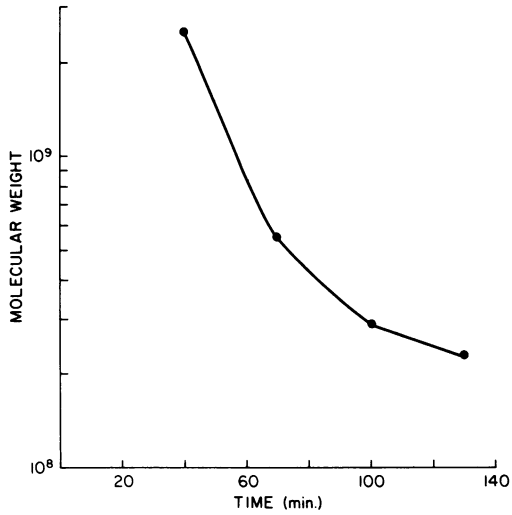

FIG. 1. Effect of sonication at different time periods upon particle size of purified group A *Streptococcus* cell wall fragments (PG-APS), measured by light scattering.

The PG-APS fragments derived by sonication were also separated by differential centrifugation in PBS into three populations, labeled 100s60 (smallest), 100p60 (intermediate), and 10p30 (largest). When these isolated fractions of different particle size were placed on sucrose gradients, they sedimented through the gradient at discrete rates and as relatively homogeneous peaks (Fig. 4).

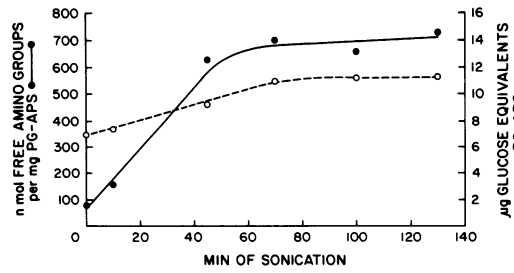

FIG. 2. Effect of sonication for different time periods on peptide cross-linking of PG-APS fragments as measured by the increase in terminal amino acids and effect on average length of glycan polymers as measured by the increase in reducing groups.

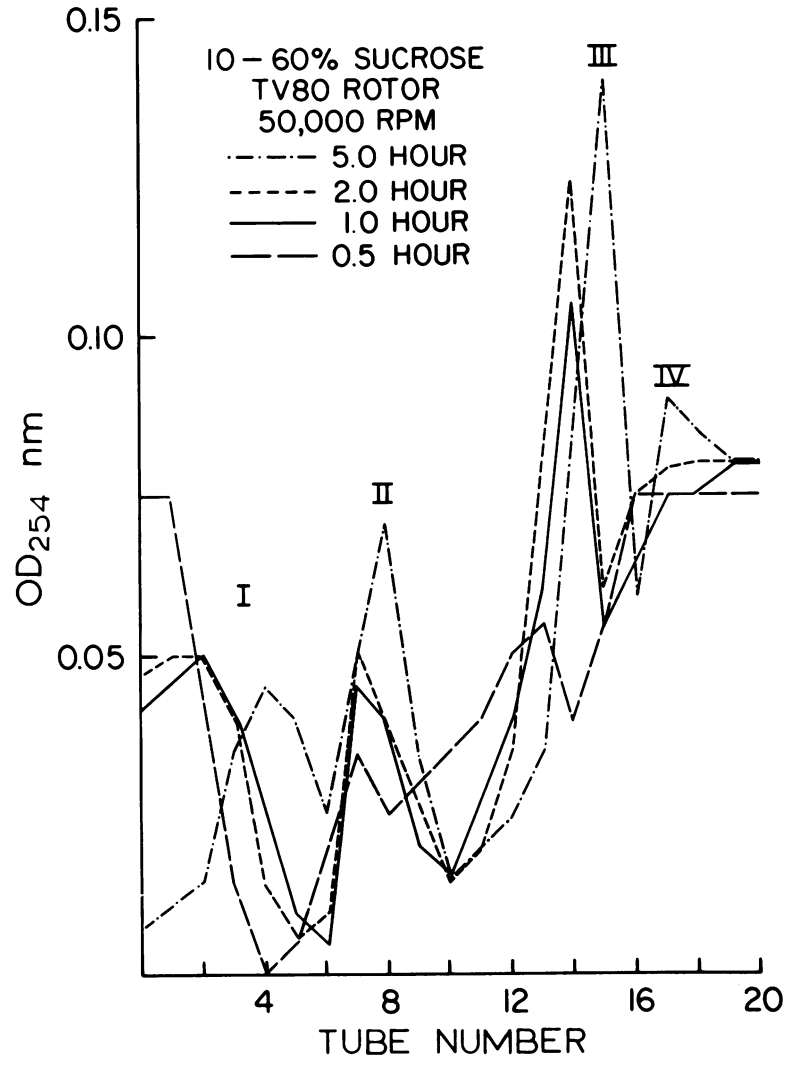

FIG. 3. Heterogeneity of the particle size of PG-APS fragments resolved by velocity sedimentation in a sucrose gradient. The PG-APS suspension was sonicated for 70 min and placed on a 10 to 60% gradient. Density increases with tube number. OD_{254} nm, Optical density at 254 nm.

The size distribution of the PG-APS fragments separated by differential centrifugation was further characterized by filtration on Bio-Gel A-150 (Fig. 5). Most of the 100p fragments of PG-APS were excluded on this gel, indicating a molecular weight in excess of 150×10^6. Most of the 100s fragments were retained on the gel to give an estimated size range of 4×10^6 to 150×10^6 daltons, with the most prominent population estimated to be 4×10^6 to 6×10^6 daltons. When examined by light scattering, the molecular weight of the 100s60 fraction was calculated to be 5.3×10^6, in close agreement with gel filtration. The light scattering value for the 100p60 fraction was 50×10^6 daltons, one-third the value of the gel filtration estimate. The chemical analysis of these fractions is shown in Table 1. The free amino group concentration was 700 nM/mg for 100s60 and 480 nM/mg for the 100p60 fraction. The concentration of reducing groups, such as glucose, was 11.5 μg/mg for 100s60 and 9.4 μg/mg for the 100p60 fraction.

Relationship of particle size of PG-APS fragments to arthropathogenicity. To determine which fragment size was most effective in producing experimental arthritis, three groups of rats were injected i.p. with equivalent doses (based upon rhamnose) of each PG-APS fraction suspended in PBS. The results of one experiment are illustrated in Fig. 6, and a summary of

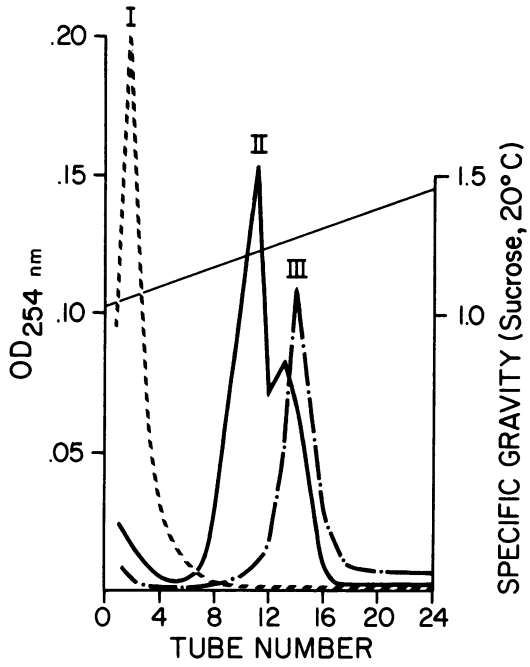

FIG. 4. Velocity sedimentation of three fractions of PG-APS obtained by differential centrifugation. The PG-APS suspension was centrifuged for 2 h on a 10 to 60% sucrose gradient.

three experiments is recorded in Table 2. The number of rats in each group was limited by the relatively low yield of the 10p and 100s fractions compared with the 100p fraction and by the toxicity of the 10p fraction. In experiments 1 and 2, three of eight rats injected with the 10p fraction died within 24 h.

In each of the experiments, the smallest fragments (100s) produced the most severe acute inflammation, but relatively less of the late, recurrent, chronic joint disease. In contrast, the intermediate-sized fragments (100p) produced moderate acute inflammation, but the most severe chronic, degenerative disease. In experiments 1 and 3, the maximum chronic score produced by the 100p fraction was significantly different ($P \leq 0.05$) from that produced by 100s. In experiment 1, the acute inflammation produced by 100s was significantly different from that produced by 100p. In experiment 3, each fraction was tested at three concentrations. The greater capacity of the 100s fraction to produce acute joint inflammation compared with the 100p and 10p fractions was most evident with the lower doses ($P \leq 0.001$). None of the fractions produced persistent joint lesions with the lower doses.

The largest fragments (10p) produced negligible acute inflammation, and the joints appeared negative by clinical inspection over the first 5 to 9 weeks after injection. Arthritis did become apparent in some rats by 40 days in experiment 1, by 68 days in experiment 2, and by 41 days in experiment 3. The 10p fraction in experiment 3 differed from the other 10p preparations in that after resuspension in PBS, it was centrifuged at 3,000 × *g* for 30 min to remove the largest PG-APS particles. Since these particles were the least effective in inducing inflammation, their removal left a more arthropathic preparation per microgram of rhamnose injected.

The relative severity of the chronic, erosive synovitis and bone destruction occurring in the late stages of disease was confirmed by the radiographic scores measured at the termination of the experiments (Table 2).

Arthropathic properties of isolated PG. In the first experiment, 10 rats were injected i.p. with

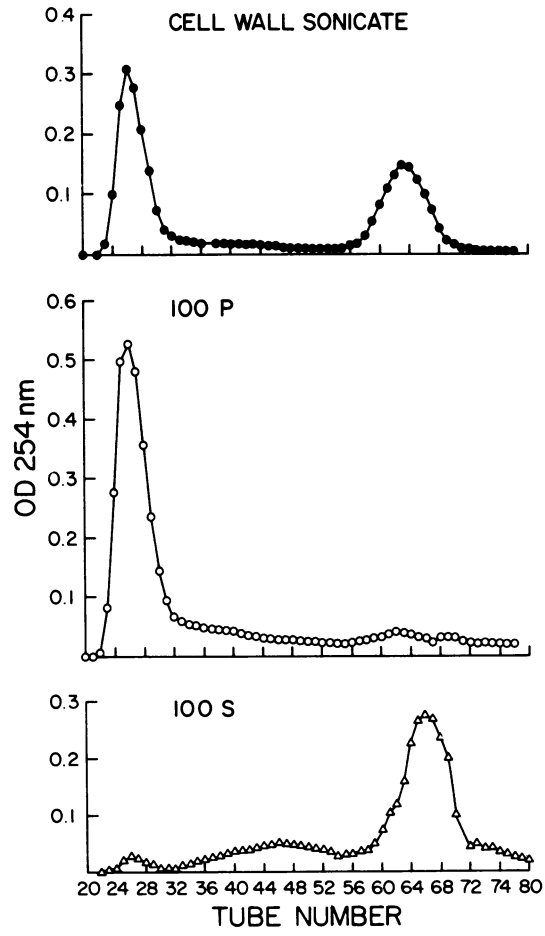

FIG. 5. Size distribution on Bio-Gel A-150 of PG-APS fragments in the whole suspension of sonicated PG-APS and in the 100p and 100s fractions separated by differential centrifugation.

TABLE 1. Composition of cell wall (PG-APS) fractions

Component[a]	Cell wall (%)[b]	PG-APS fractions (%)[c]		
		10p30	100p60	100s60
Rhamnose	24.9	18.8	25.2	23.4
Glucose	0	0	0	0
Glucosamine	14.0	9.8	11.9	11.3
Muramic acid	5.1	3.4	4.9	3.8
Lysine	ND[d]	5.0	5.2	5.2
Glutamic acid	ND	5.7	6.1	6.8
Alanine	ND	12.9	16.4	14.1

[a] Other amino acids, neutral sugars, and amino sugars which were detected total less than 2% of the lyophilized dry weight.

[b] Percentage of lyophilized dry weight.

[c] Fractions derived by differential centrifugation from cell wall preparation treated with ribonuclease, trypsin, and papain and extracted with chloroform-methanol. Percentage of lyophilized dry weight.

[d] ND, Not determined.

7.5 mg of PG per rat from group A cell walls suspended in PBS. Three of the rats died between 6 and 18 h, indicating that this was an excessive dose for assessment of arthropathic activity. Four of the surviving seven rats developed moderate acute inflammation in the joints of the ankles and feet, consisting of erythema and edema, which reached a peak at 3 days after injection and rapidly subsided. The average joint score at day 3 was 1.6 (out of a possible score of 16), and all rats were negative by clinical criteria 10 days postinjection. There was no evidence of any recurrence of joint inflammation over a period of 2 months. All rats were X-rayed at 43 days postinjection, and no radiographic evidence of arthritis was detected.

The question of the arthropathic capacity of PG, as opposed to PG-APS, is important for pursuing the identification of the minimal effective structure. Therefore, further experiments were done to determine whether PG derived from another strain of *Streptococcus* and tested in different doses and fragment sizes might be more effective as an arthropathic agent. PG isolated from strain K-43 group A variant streptococci was sonicated and injected as described in Materials and Methods. None of the rats injected with the lower doses of 1.0 or 0.3 mg developed any detectable joint inflammation. Moderate acute inflammation of the ankles and feet developed in the rats injected with 3.0 mg of PG samples which were not sonicated (average score, 2.3; 6 of 6 positive) or were sonicated for only 18 s (average score, 2.8; 3 of 6 positive). These responses reached a peak 3 days after injection and rapidly subsided. Samples of PG sonicated for 30 s or longer induced only a slight, transient erythema in the feet over the first 48 h in 50% of the rats. Recurrent, destructive, chronic joint inflammation was not detectable by clinical or radiographic assessment in any rats over an observation period of 6 weeks after a single i.p. injection of an aqueous suspension of any of these preparations of PG.

DISCUSSION

The covalently bound complex of PG-APS polymers is the etiological agent of experimental arthritis induced by the systemic injection of bacterial cell wall fragments in aqueous suspension (4a, 16). The progressive destruction of joints is associated with the localization and persistence of PG-APS in joint tissue (6, 7). The outstanding features of this cell wall structure are the toxicity and capacity to invoke dysfunction of the immune system, combined with

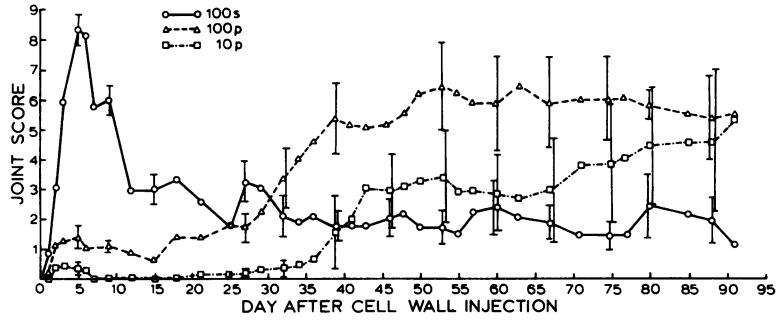

FIG. 6. Effect of particle size of PG-APS fragments isolated from group A streptococcal cell walls upon acute and chronic joint diseases. All rats were injected i.p. with PG-APS fragments (30 μg of rhamnose per g of body weight. □ (large fragments), 5 rats; △ (intermediate fragments), 12 rats; ○ (small fragments), 6 rats. Mean joint score ± standard error of the mean. Total of four legs; maximum score is 16. The level of significance between the three groups was calculated for each day that scores were recorded. For clarity, the standard error of the mean is only shown at 5-day intervals. Fraction 100s60 is significantly greater than 100p60 or 10p30 at days 2 through 18 and significantly less than fraction 100p from days 38 to 92 ($P \leq 0.05$).

TABLE 2. Summary of the effect of particle size and dose of PG-APS fragments on acute and chronic joint disease

Expt	Fraction	Dose[a]	Acute joint lesions			Chronic joint lesions			Radiographic assessment	
			Score[b]	P[c]	No. positive/ total[d]	Score[b]	P[c]	No. positive/ total[e]	Score[f]	Day
1	100s	30	8.9 ± 0.5	0.0001	6/6	2.7 ± 0.7	NS[g]	5/6	1.2 ± 0.7	97
	100p	30	2.0 ± 0.4	0.02	12/12	7.8 ± 1.5	NS	12/12	5.3 ± 2.2	97
	10p	30	0.45 ± 0.3		2/5	5.6 ± 1.9		5/5	4.1 ± 2.7	97
2	100s	30	10.25 ± 1.3		10/10	7.8 ± 2.1	NS	7/10	3.0 ± 1.0	97
	100p	30	8.25 ± 1.2		10/10	8.2 ± 1.8	0.05	10/10	5.0 ± 1.5	97
	10p	30	0		0/5	2.6 ± 1.8		1/5	2.1 ± 2.0	97
3	100s	30	11.2 ± 0.4		8/8	5.2 ± 1.2	0.025	7/8	1.6 ± 0.4	62
		10	4.25 ± 0.6	0.00001	8/8	0		0/8	ND[h]	
		3	0.56 ± 0.2		4/8	0		0/8	ND	
	100p	30	9.6 ± 0.8	0.00002	8/8	9.3 ± 1.8	0.002	7/8	3.6 ± 1.0	62
		10	0.59 ± 0.2	0.04	4/8	0		0/8	ND	
		3	0		0/8	0		0/8	ND	
	10p	30	4.8 ± 0.8		8/8	1.8 ± 1.0		4/8	0.13 ± 0.1	62
		10	0.12 ± 0.09		1/8	0		0/8	ND	
		3	0		0/8	0		0/8	ND	

[a] Micrograms of rhamnose per gram of body weight, injected i.p. Each experiment was done with separate batches of cell wall.

[b] Mean ± standard error of maximum joint scores recorded for acute inflammation (day 3 to 7 after cell wall injection) or for chronic joint disease (day 19 to termination) by clinical evaluation.

[c] Level of significance compared with 10p fraction.

[d] Number of rats with clinically apparent joint inflammation/total number of rats injected.

[e] Number of rats with clinically evident recurrence of joint disease/total number of rats injected.

[f] Mean score of hind joints ± standard error of the mean at indicated day of termination.

[g] NS, Not significant.

[h] ND, Not determined.

resistance to biodegradation (1, 17, 19). Although the toxic properties of this complex are associated with the PG moiety (1, 10, 11), as shown in this report, a single injection of the isolated PG in aqueous suspension cannot, by itself, induce chronic erosive arthritis. This is consistent with studies of other models in which PG in aqueous suspension could induce acute injury but not chronic inflammatory disease (1). The modulation of toxicity by the polysaccharide moiety is related to the rapid degradation of PG by tissue enzymes in contrast to the persistence of the PG-APS complex (13, 17, 19).

Since the isolated PG is not effective, we must examine the PG-APS complex to define the minimal structural unit of cell wall which can induce and maintain a chronic inflammatory process. The standard preparation of PG-APS which is used to induce arthritis in rats is sonicated for 70 min, which yields a polydisperse suspension of fragments. This suspension was resolved into discrete populations by velocity sedimentation, which means that the peaks represent families of different particle size rather than particles of different densities. Equilibrium separation, reflecting density differences, would have meant variable contamination of fragments with material such as membrane lipids.

The chemical analysis of the fractions separated by differential centrifugation showed relatively small differences in composition, which provides further evidence that they represent three populations of PG-APS fragments differing primarily in average particle size.

The smaller fragments of PG-APS, with the predominate species having a molecular weight of 5.3×10^6, induced the early acute phase of joint inflammation with relatively less severe chronic disease. Larger fragments, with an average molecular weight of 500×10^6, induced a minimal early phase but did induce a chronic arthritis of late onset. Intermediate-size fragments, with a molecular weight of approximately 50×10^6 (by light scattering), induced both acute and chronic phases of the disease. Thus, instead of identifying a minimal unit of PG-APS responsible for the bimodal acute and chronic arthritis, this study demonstrates that fragments of different size are responsible for different phases of the clinical pattern of the experimental joint disease.

The fragment size is a reflection of the length

of glycan polymers (measured as reducing sugar) and the extent of peptide cross-linking (measured as free amino groups). The larger number of free peptide side chains in the 100s fragments, indicated by the higher concentration of free amino groups, could be important in initiating acute inflammation. Further definition of the fine-structure properties of PG-APS required to initiate and to maintain inflammation of the joints is being pursued with muralytic and endopeptidase enzymes (C. Chetty and J. H. Schwab, manuscript in preparation). Another effect of size could be on transport and elimination. In support of this explanation, quantitative measurement of PG-APS in tissues of rats demonstrates that the different patterns of arthropathogenesis, induced with fragments of different size, correlate with differences in tissue distribution and the rate of elimination (R. A. Eisenberg, A. Fox, S. K. Anderle, R. R. Brown, and J. H. Schwab, manuscript in preparation). The practical significance of these observations is that to reproduce this experimental model of arthritis the distribution of particle sizes within a preparation of PG-APS fragments is very important. Thus, if a disproportionate amount of the suspension consists of large fragments, joint disease will not appear until several weeks after injection, and the incidence may be low. Similar results were obtained in other experimental models in which the size of cell wall fragments was an important determinant of disease (14, 15, 18).

The observations reported here are consistent with the concept that the extent of degradation of bacterial cells by the host may be an important factor determining the arthropathogenicity of the residual bacterial cell wall debris. We hypothesize that some individuals are more susceptible because less effective degradation of bacterial cells results in the accumulation of sufficient quantities of toxic persistent debris, with the consequent development of chronic inflammation.

ACKNOWLEDGMENTS

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