LIPOPOLYSACCHARIDE INDUCES RECURRENCE OF ARTHRITIS IN RAT JOINTS PREVIOUSLY INJURED BY PEPTIDOGLYCAN-POLYSACCHARIDE

BY STEPHEN A. STIMPSON,* RONALD E. ESSER,* PHILIP B. CARTER,§ R. BALFOUR SARTOR,‡ WILLIAM J. CROMARTIE,* AND JOHN H. SCHWAB*

From the Departments of *Microbiology and Immunology, and ‡Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514, and the §Department of Microbiology, Pathology, and Parasitology, School of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606.

LPS and peptidoglycan or covalent peptidoglycan-polysaccharide (PG-PS)[1] complexes are the major toxic components of bacterial cell walls. Lipid A and PG possess many of the biologic activities that LPS and PG-PS, respectively, have in common, such as pyrogenicity, polyclonal activation of lymphoid cells, complement activation, mitogenicity, macrophage activation, and adjuvanticity (1–3). Both also induce arthritis after injection into laboratory animals. Systemic injection of rats with a sterile, aqueous suspension of PG-PS from the group A streptococcus (PG-APS) induces a chronic erosive relapsing arthritis that has many features of rheumatoid arthritis (4–6). Distinct PG-PS structures from a number of other bacteria, including human indigenous intestinal species, induce arthritis of varying chronicity (7–10). LPS induces primarily an acute arthritis of relatively short duration after intraarticular (i.a.) injection (7, 11–15), but there have been few reports of its arthropathic activity after systemic administration (16, 17, 17a).

In spite of the shared biologic properties and ubiquitous distribution of LPS and PG-PS, the interaction of their phlogistic activities in an inflammatory process has received little attention. During investigations of mechanisms of recurrent arthritis induced by PG-APS, we noted that a systemic injection of 100 µg of *Salmonella typhimurium* LPS induced a transient recurrence of arthritis in rat joints previously inflamed by exposure to PG-APS (7,18). We now report that the systemic injection of a much lower dose of LPS will induce a recurrence of arthritis in joints inflamed 3 wk previously by the intraarticular injection of PG-APS and describe this reaction in detail. We show that LPS from many bacteria, including *Yersinia enterocolitica* and *Neisseria gonorrhoeae*, are active; that lipid A

[1] *Abbreviations used in this paper:* APS, group A streptococcus polysaccharide; i.a., intraarticular; PG, peptidoglycan; PS, polysaccharide.

is the active moiety; and that the reaction is influenced by the genetic background of the rat. We provide evidence that the reaction is dependent upon the persistence of high-molecular-weight PG-APS in the joint, and that T lymphocytes are not required. In addition, we show that, in ~50% of naive, previously uninjected rats, a high systemic dose of LPS induces a mild, transient acute synovitis. These studies support the concepts that LPS and peptidoglycan can interact in an inflammatory process and that LPS may play a role in recurrent episodes of rheumatoid arthritis or in reactive arthritis.

Materials and Methods

Source of LPS, PG-APS, and Other Reagents. S. *typhimurium* wild type strain LT-2 LPS, *Escherichia coli* strain 0111:B4 LPS, and S. *typhimurium* Re mutant strain G30/C21 LPS were purchased from Ribi Immunochem Research, Inc., Hamilton, MT. S. *typhimurium* W LPS was purchased from Difco Laboratories, Detroit, MI. *N. gonorrhoeae* strain FA 5100 LPS (19) was a gift from W. M. Shafer, Emory University, Atlanta, GA. *Y. enterocolitica* strain WA LPS was a gift from R. R. Brubaker, Michigan State University, East Lansing, MI. S. *minnesota* Re mutant strain R595 LPS and *E. coli* Re mutant strain 515 lipid A were gifts from H. Brade, Forschungsinstitut, Borstel, Federal Republic of Germany.

PG-APS was prepared by the SDS extraction of cell walls from *Streptococcus pyogenes*, group A, strain D-58, as described in detail previously (10). All of the experiments reported here were done with a fraction of sonicated PG-APS fragments that sediment at 100,000 g but not at 10,000 g (the 100P fraction). Fragments in this fraction have an average mol wt of 5 × 10^7 (20).

Polymyxin B sulfate (8,156 U/mg) was purchased from Sigma Chemical Co., St. Louis, MO.

Mutanolysin (*N*-acetylmuramidase) was purchased from Miles Laboratories, Inc., Elkhart, IN. It was dissolved in PBS, pH 7, with brief sonication before injection into rats, as described previously (21).

Sterility of preparations for injection into rats was monitored by streaking on blood agar plates.

Experimental Animals and Induction of Arthritis. Unless otherwise noted, experiments were done with female rats, age 6–8 wk, purchased from the following sources: outbred Sprague-Dawley rats from Zivic-Miller Laboratories, Allison Park, PA; inbred Lewis rats from Charles River Breeding Laboratories, Wilmington, PA; and inbred Buffalo rats from Simonsen Laboratories, Bilroy, CA.

Athymic Lewis rats (LEW *rnu/rnu*) and their euthymic (*rnu/+*) (22, 23) littermates were produced by the School of Veterinary Medicine, North Carolina State University, Raleigh, NC. The rats were obtained by backcrossing Rowett outbred nude rats onto LEW/N females for over five generations. The animals were housed in filter-top cages and fed autoclaved fortified rat chow and water.

LPS was dissolved in distilled H_2O, with gentle sonication if necessary, and diluted to the desired concentration in PBS. PG-APS was also suspended in PBS. Before injection, LPS and PG-APS preparations were gently sonicated for 3 min in a 9 KHz Sonic Oscillator (Raytheon Co., Waltham, MA) to promote even dispersion. Rats were injected under ether anesthesia. Intravenous injections (0.5 ml) were given in the tail vein. Intraarticular injections (10 μl) were given in the ankle joint, through the Achilles tendon just proximal to the calcaneous, using a 25-gauge needle adapted to a micropipette (7, 15). Injection dosages and times are indicated in the Results. Typically, PG-APS (5 μg) or LPS (10 μg)

TABLE I

Arthritis Induced by Systemic Injection of LPS into Naive (Previously Uninjected) Rats

Exp.	LPS	Rat		Dose		Mortality*	Arthritis 48 h after LPS injection	
		Average age	Average weight	Micro-grams per rat	Micro-grams per gram body wt		Incidence‡	Mean joint score (± SEM)§
		wk	*g*					
1	*S. typhimurium* LT-2 LPS	6	130	50	0.39	6/11	2/5	1.0 ± 0.5
1		9	222	50	0.23	2/9	3/7	0.9 ± 0.4
1		16	364	50	0.14	0/9	3/9	1.3 ± 0.4
1		28	412	50	0.12	0/9	5/9	1.4 ± 0.4
2	*S. typhimurium* G30/C21 Re mutant LPS	8	150	100	0.67	0/7	5/7	1.5 ± 0.3
2	*S. minnesota* R595 Re mutant LPS	8	150	100	0.67	0/7	0/7	0

LPS was injected intravenously into Sprague-Dawley rats. Control rats injected with PBS never developed arthritis.
* Number of rats that died/number of rats injected. All deaths occurred within 24 h of LPS injection.
‡ Number of rats having a joint score of at least one/number of surviving rats.
§ Includes rats that did not develop arthritis.

was injected in right ankles, and PBS (10 µl) was injected in left ankles. 3 wk later, rats received an intravenous injection of LPS or PG-APS. In some experiments, LPS was injected intravenously into naive, previously uninjected rats.

The severity of arthritis was scored grossly on a scale of 0 (no apparent inflammation) to 4 (severe inflammation) based on erythema and edema of the periarticular tissues and enlargement of the joints (4, 10). In several experiments, as indicated in the Results, the presence of synovitis was confirmed by histologic study of joint sections. Rats were killed with CO_2 and ankle joints were removed, skinned, fixed in formalin, decalcified, embedded in paraffin, sectioned, and stained with H and E (4).

FACS Analysis of Splenocytes from rnu/rnu and rnu/+ Rats. All mAbs were purchased from Seralab (distributed by Accurate Chemical and Scientific Corp., Westbury, NY) except MRC OX-1 and MRC OX-42, which were prepared as culture supernatants at the North Carolina State University FACS/Hybridoma Facility, School of Veterinary Medicine, North Carolina State University, Raleigh, NC using clones obtained from A. F. Williams, Oxford (OX-1) and S. Brostoff, Charleston, SC (OX-42). The mouse anti-rat leukocyte mAbs recognize the following determinants: MRC OX-1, a monomorphic leukocyte common antigen (24); MRC OX-19, all thymocytes and peripheral T cells, and a subset of B cells (25); MRC OX-8, most thymocytes, the cytotoxic/suppressor T cell subset, and the majority of natural killer cells (26, 27); W3/25, most thymocytes, the helper T cell subset, and macrophages (26, 28, 29, 30); MRC OX-7, a monomorphic Thy-

FIGURE 1. Histologic appearance of ankle joints following systemic injection of LPS. (*A*) Acute synovitis, with an exudate containing polymorphonuclear leukocytes (PMNs) and fibrin in the joint space, 48 h after intravenous injection of 50 µg of *S. typhimurium* LT-2 LPS in a naive, previously uninjected Sprague-Dawley rat (×120). All other sections (*B–F*) are from Lewis rats 3 d after injection with various LPS preparations. 3 wk before LPS injection, the right ankle joints of these rats had been injected intraarticularly with 5 µg of PG-APS, and the left joints had been injected with PBS. (*B*) PG-APS-injected joint after intravenous injection with 25 µg of *N. gonorrhoeae* LPS, showing a severe acute exudative inflammatory reaction. Note the PMNs and fibrin in the joint space (×120). (*C*) PBS-injected joint of the rat described in (*B*). The joint space is clear, and no inflammation is seen (×120). (*D*) PG-APS-injected joint after intravenous injection with 25 µg of *Y. enterocolitica* LPS, showing PMNs and some mononuclear cells in the joint space and the synovial stroma. Hyperplasia of the synovial lining cells is also seen. (×540). (*E*) PG-APS-injected joint after intravenous injection with 25 µg of *S. typhimurium* Re mutant LPS, showing an acute exudative synovitis similar to that seen in (*B*) and (*D*) above (×120). (*F*) PG-APS-injected joint after intravenous injection of a mixture of 25 µg of *S. typhimurium* Re mutant LPS and 250 µg of polymyxin B. No acute inflammation is seen (×120).
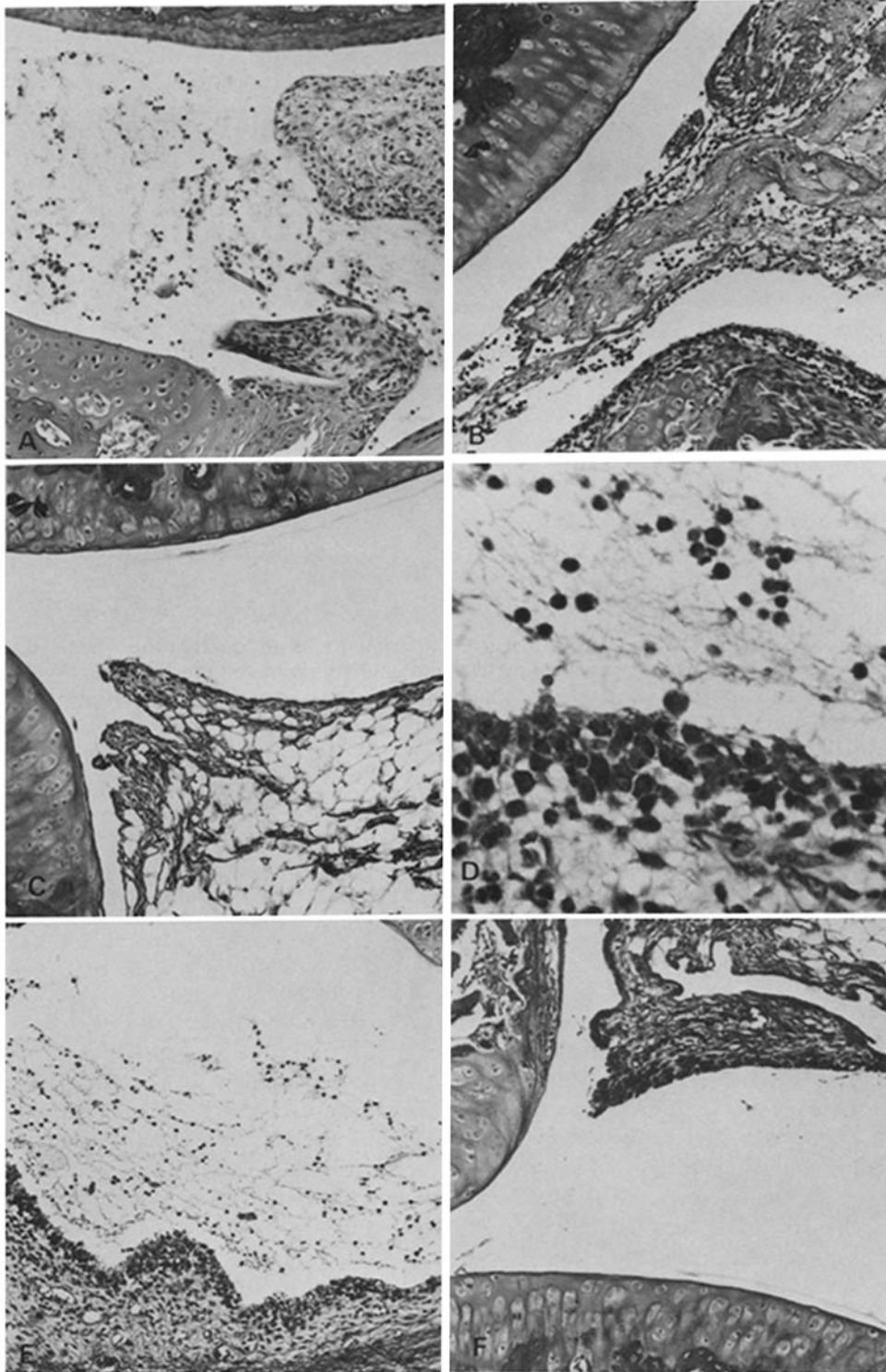
1 determinant on thymocytes and immature lymphocytes (31, 32); MRC OX-12, κ light chains (33); MRC OX-6, a monomorphic Ia determinant (34); and MRC OX-42, a complement receptor type 3 determinant on macrophages, granulocytes, and dendritic cells (35). Splenocytes were analyzed on a FACS 440 (Becton Dickinson, Mountain View, CA), essentially as described by White, et al. (30). Briefly, splenocyte suspensions were prepared in RPMI-1640 containing 10 mM HEPES with no FCS or antibiotics (RPMI). mAb was added to aliquots of cells and incubated at 4°C for 30 min. After washing, cells were resuspended in FITC-conjugated goat anti–mouse IgG (FITC–anti–mouse IgG) (Cappel Laboratories, West Chester, PA) and incubated at 4°C for 30 min. The FITC–anti–mouse IgG was diluted in 10% rat serum even though it was said to have no crossreactivity with rat antigens. Finally, splenocytes were washed, resuspended in RPMI, and analyzed. Controls included splenocyte suspensions incubated without mAb or with FITC–anti–mouse IgG alone. Results are expressed as the mean percent of splenocytes that are recognized by a given mAb, after subtracting the background binding by FITC–anti–mouse IgG alone. This background was usually <6% and not >8.8%.

Results

Acute Synovitis after Systemic Injection of Endotoxin in Naive Rats.   A single intravenous injection of *S. typhimurium* LT-2 LPS into rats induced a transient acute synovitis (Table I). By gross inspection, this reaction was most severe 24–48 h after injection, then quickly resolved, and was usually undetectable by day 7. Re mutant LPS from *S. typhimurium*, but not from *S. minnesota*, was also able to induce this reaction. Histologic studies revealed an acute exudative reaction, with polymorphonuclear leukocytes and small amounts of fibrin in the joint space, which was clearly evident 2 d after injection (Fig. 1 *A*). Hyperplasia of the synovial lining cells was also noted in many rats. By day 5, these reactions had subsided, and by day 14 were gone, with little or no evidence of chronic inflammation. The effect of rat age on the response to a single 50 μg i.v. dose of LPS is shown in Table I. This was a lethal dose for many of the younger rats and it induced transient diarrhea and a sickly appearance in most rats of all ages. The severity and incidence of arthritis did not change significantly with increased age, even though the dose of LPS per gram of body weight decreased. This indicated that older rats were at least as, and perhaps more susceptible to LPS-induced synovitis than younger rats.

It should be emphasized that although the presence of this reaction could easily be confirmed histologically, it was not very severe, was seen in only ~50% of rats injected with high doses of LPS, and was easily overlooked in the gross if rats were not examined carefully. Gross evidence of synovitis was noted only in the ankle joints; other joints were not examined histologically.

Recurrence of Arthritis after Systemic LPS Injection in Joints Previously Exposed to PG-APS.   Intraarticular injection of PG-APS into the rat ankle joint induced an acute course of arthritis that peaked in severity 12–24 h after injection and gradually subsided over the next 3 wk. Aside from a transient increase in joint swelling in some rats 7–10 d after injection, arthritis resolved without sponta-neous recurrence of inflammation.

Intravenous injection of LPS induced a recurrence of arthritis in joints injected 3 wk previously with PG-APS. The severity of inflammation increased to a peak 48 h after LPS injection, and then gradually resolved over the next week. No ankylosis or lasting loss of joint function was seen. The smallest dose of *S.*

TABLE II

Recurrence of Arthritis Induced by Systemic Injection of Various Types and Doses of LPS

Exp.	LPS	Dose	Arthritis 48 h after LPS injection				$p^{\ddagger}$
			PG-APS-injected (right ankle)		PBS-injected (left) ankle		
			Incidence*	Mean joint score (± SEM)	Incidence	Mean joint score (± SEM)	
		μg					
1	S. typhimurium LT-2	100	7/7	2.2 ± 0.3§	5/7	1.1 ± 0.3	<0.001
1		50	6/6	2.5 ± 0.3	4/6	1.1 ± 0.2	<0.025
2		50	8/8	2.9 ± 0.1	6/8	1.2 ± 0.3	<0.001
1		25	8/8	2.3 ± 0.2	4/8	0.6 ± 0.3	<0.001
1		10	9/9	2.1 ± 0.2	2/9	0.5 ± 0.2	<0.001
2		10	8/8	2.4 ± 0.1	3/8	0.4 ± 0.3	<0.001
3		10	7/8	2.0 ± 0.4	2/8	0.2 ± 0.1	<0.001
2		3	5/8	1.3 ± 0.3	0/8	0.1 ± 0.1	<0.005
2		1	2/8	0.8 ± 0.3	0/8	0	
2		0.3	1/8	0.5 ± 0.2	0/8	0	
2		0.1	0/8	0.5 ± 0.2	0/8	0	
4	S. typhimurium W	50	6/6ǁ	2.0 ± 0.3	0/6	0	<0.001
5	E. coli	100	8/8	2.3 ± 0.2	2/8	0.6 ± 0.2	<0.001
6	Y. enterocolitica	25	8/8	2.5 ± 0.3	2/8	0.5 ± 0.4	<0.001
6	N. gonorrhoeae	25	8/8	2.6 ± 0.2	0/8	0	<0.001

On day 0, the right ankle of each rat received an intraarticular injection of 5 μg of S. pyogenes PG-APS. The left ankle received an intraarticular injection of PBS. 3 wk later, each rat received an intravenous injection of LPS.

* Number of ankles in which joint score increased by at least one after intravenous LPS injection/ total number of ankles injected.

‡ Significance of the difference between the mean joint score of PG-APS-injected vs. PBS-injected joints (*t* test for paired samples).

§ Immediately before injection of LPS, the mean score of PG-APS-injected joints was never >0.4 ± 0.2, and PBS-injected joints showed no apparent inflammation. Mean joint scores for PG-APS-injected ankles 48 h after LPS injection were significantly higher than those immediately before LPS injection, except for the 1, 0.3, and 0.1 μg doses (*p* < 0.005, *t* test for paired samples).

ǁ Arthritis 72 h after LPS injection.

typhimurium LT-2 LPS that induced a recurrence of arthritis in 100% of PG-APS-injected joints was 10 μg, and a recurrence was induced with as little as 300 ng (Table II). LPS preparations from *E. coli*, *Y. enterocolitica*, and *N. gonorrhoeae* were also active (Table II).

Representative clinical courses of arthritis in Lewis and Buffalo rats are shown in Fig. 2 *B*. Arthritis induced by intraarticular injection of PG-APS was less severe in Buffalo than in Lewis rats. In addition, the recurrence of arthritis induced by the intravenous injection of LPS in Buffalo rats was of lower incidence, severity, and duration than in Lewis rats (Fig. 2 *B*).

Histological studies were done to confirm and extend the clinical observations. Immediately before intravenous injection of LPS, joints injected 21 d previously
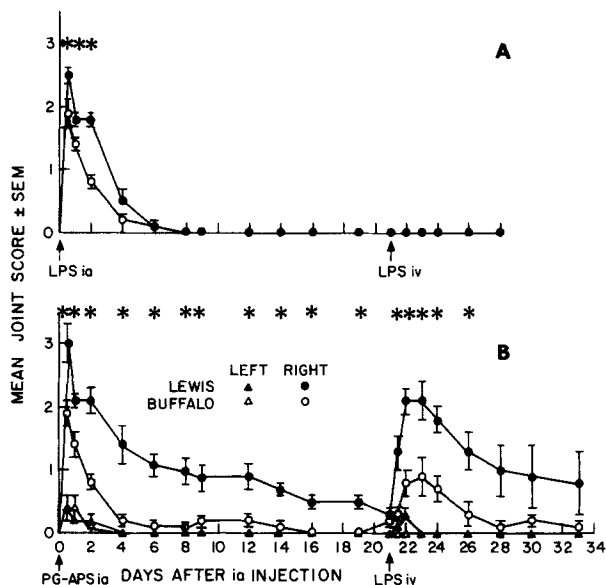
FIGURE 2. Clinical course of arthritis induced in Lewis or Buffalo rats. Asterisks indicate times at which the right ankle mean joint score for Lewis rats was significantly higher than that for Buffalo rats (t test for independent means, $p < 0.01$). (A) The right ankle joint of each of 14 Lewis (●) and 14 Buffalo (○) rats was injected intraarticularly with 10 μg of S. typhimurium LT-2 LPS. Left ankles received PBS (not shown). On day 21, each strain was injected with 25 μg of S. typhimurium LT-2 LPS (seven rats) or Y. enterocolitica LPS (seven rats). No inflammation was noted in left or right ankle joints after this injection. (B) The right ankle joint of each of eight Lewis (●) or Buffalo (○) rats was injected intraarticularly with 5 μg of PG-APS. Left ankles received PBS (▲, Lewis; △, Buffalo). On day 21, all rats were injected intravenously with 25 μg of S. typhimurium LT-2 LPS.

with PG-APS had little or no evidence of acute inflammation, but did have some residual mild chronic synovitis. This consisted of hyperplasia of the synovial lining cells, fibrosis of the synovial stroma, diffuse and focal collections of lymphocytes and macrophages, and limited pannus formation and marginal erosion of the articular cartilage.

2 d after intravenous injection of LPS, a severe acute inflammatory exudate, consisting primarily of polymorphonuclear leukocytes, fibrin, and some erythrocytes, was seen in PG-APS-injected joints (Fig. 1, B–D). This was not seen in PG-APS-injected joints of control rats given PBS instead of LPS.

By 8 d after injection of 10 μg of S. typhimurium LPS, the acute reaction in PG-APS-injected joints had resolved. Mild chronic inflammation, similar to that seen immediately before LPS injection, persisted and was still evident at 20 d. In rats given a higher dose (100 μg) of E. coli LPS, the chronic inflammation in PG-APS-injected joints 9 and 20 d after LPS injection was clearly more severe than that at 2 d. In addition, a mild acute exudative reaction was still evident at these times, suggesting an ongoing inflammatory process.

Acute synovitis following high-dose LPS injection was seen in contralateral ankle joints initially injected with PBS. For clarity, this reaction is shown only in Table II and Fig. 2B. This reaction was similar histologically and clinically to that induced by LPS in naive rats (Table I, Fig. 1A), except that it was usually most severe 24 h after LPS injection. It was consistently of lower incidence, severity, and duration than that seen in ankle joints previously exposed to PG-APS.

Recurrence of Arthritis after Systemic Injection of Re Mutant LPS or Lipid A. The ability of an intravenous injection of Re mutant LPS or lipid A to induce a

TABLE III

Recurrence of Arthritis Induced by Systemic Injection of Re Mutant LPS or Lipid A

Exp.	Endotoxin	Rat strain	Dose	Arthritis in PG-APS-injected (right) joint 48 h after endotoxin injection	
				Incidence*	Mean joint score (± SEM)
			μg		
1	S. typhimurium LT-2 LPS	Sprague-Dawley	10	7/7	2.6 ± 0.3 ($p < 0.02$)‡
1	S. typhimurium G30/C21 Re mutant LPS		10	6/8	1.6 ± 0.3
2			50	5/8	1.3 ± 0.2
3		Lewis	25	7/9	1.9 ± 0.4 ($p < 0.005$)‡
3	S. typhimurium Re mutant LPS mixed with 250 μg polymyxin B		25	1/7	0.3 ± 0.2
4	E. coli Re mutant (strain 515) lipid A	Sprague-Dawley	50	4/8	1.4 ± 0.3
4	S. minnesota R595 Re mutant LPS		50	8/8	2.3 ± 0.2
1			50	4/10	1.0 ± 0.2
1			10	4/10§	0.7 ± 0.2

* As for Table II.

‡ Significance of the difference between this score and the next one listed (t test for independent means).

§ Arthritis 24 h after endotoxin injection.

recurrence of arthritis in a joint previously exposed to PG-APS was examined (Table III). In general, Re mutant LPSs were less active on a dry weight basis than wild type LPS, and results were more variable. Nevertheless, they clearly possessed some activity. Interestingly, the gonococcal LPS (Table II) is similar to Re mutant LPS in that it contains only lipid A and 2-keto-3-deoxyoctonic acid (19), yet it was one of the most highly arthropathic LPSs tested. E. coli lipid A was weakly active (Table III). Mixture of polymyxin B with Re mutant LPS immediately before injection significantly reduced the incidence, severity, and duration of recurrent arthritis (Table III, Fig. 1, E and F).

Arthritis after Intraarticular Injection of LPS. Intraarticular injection of 10 μg of S. typhimurium LT-2 LPS induced an acute course of arthritis that peaked in severity ~12 h after injection and then rapidly resolved to grossly undetectable levels by day 6 (Fig. 2A). This reaction was significantly more severe in Lewis rats than in Buffalo rats. Injection of 25 μg of S. typhimurium LT-2 or Y. enterocolitica LPS i.v. 3 wk after the intraarticular LPS injection did not induce a recurrence of arthritis in either rat strain (Fig. 2A). In another experiment, 100 μg of E. coli LPS injected intravenously 3 wk after intraarticular injection of 10 μg of E. coli LPS did not induce gross or histologic evidence of arthritis in LPS-injected or contralateral PBS-injected joints (data not shown).

Injection of 300 μg of PG-APS intravenously, which will induce a recurrence of arthritis in a joint previously injected intraarticularly with PG-APS (7) did not induce a recurrence of arthritis in joints injected intraarticularly 3 wk earlier with 10 μg of S. typhimurium LT-2 LPS (data not shown).
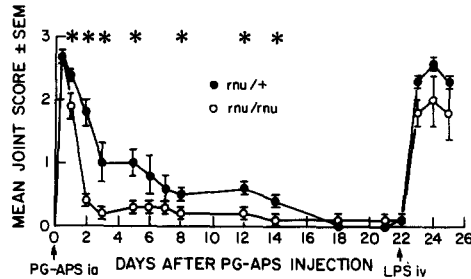
FIGURE 3. Clinical course of arthritis induced in rnu/+ (euthymic, ●) and rnu/rnu (athymic, ○) rats. The right ankle joint of each of five rnu/+ and five rnu/rnu rats was injected intraarticularly with 5 μg of PG-APS. Left ankles received PBS (not shown). On day 21, each rat was injected intravenously with 25 μg of S. typhimurium LT-2 LPS. Asterisks indicate times at which the mean joint score for rnu/+ rats was significantly higher than that for rnu/rnu rats (t test for independent means, p < 0.025).

TABLE IV

FACS Analysis of Splenocyte Subsets from rnu/+ and rnu/rnu Rats

Rat strain	Mean percent (± SEM) of splenocytes recognized by:*							
	MRC OX-1	MRC OX-19	MRC OX-8	W3/25	MRC OX-7	MRC OX-12	MRC OX-6	MRC OX-42
rnu/+	96.4 ± 0.2	37.8 ± 1.0	23.6 ± 0.4	23.2 ± 0.5	14.0 ± 0.9	37.0 ± 0.5	23.6 ± 0.9	18.6 ± 2.9
rnu/rnu	93.2 ± 0.4	1.4 ± 0.9	24.6 ± 0.8	6.4 ± 0.9	18.6 ± 0.7	45.6 ± 1.3	32.0 ± 3.0	35.0 ± 3.2‡
p§	<0.001	<0.001	NS	<0.001	<0.005	<0.001	<0.025	<0.005

* Splenocytes from five rnu/+ and five rnu/rnu rats from the experiment shown in Fig. 3. These figures take into account controls for the binding of FITC-conjugated goat anti-mouse IgG alone, for which the mean was usually <6% and never >8.8%.
‡ This mean is based on four rats.
§ Significance of the difference in the mean for rnu/+ vs. rnu/rnu rats (t test for independent means).

LPS-induced Recurrence of Arthritis in Athymic Rats. The role of T lymphocytes in LPS-induced recurrence of arthritis was examined by comparing the responses of rnu/rnu (athymic) and rnu/+ (euthymic) rats (Fig. 3). The intraarticular injection of PG-APS induced acute arthritis in both strains, but arthritis in rnu/rnu rats subsided more quickly than that in rnu/+ rats. A recurrence of arthritis was induced in both rat strains by the intravenous injection of LPS 3 wk after intraarticular PG-APS injection. There was no difference in the course of arthritis in male (data not shown) and female rnu/+ rats. All rats were sacrificed 3 d after intravenous LPS injection for histological study of joints and FACS analysis of splenocytes. A severe acute inflammatory exudate in the right ankle joint of every rat was observed, and no significant differences between rnu/rnu and rnu/+ rats were seen.

Splenocytes from the female rats were compared by FACS analysis to confirm the T cell deficiency of rnu/rnu rats. As shown in Table IV, splenocytes from rnu/rnu rats were severely deficient in cells recognized by the MRC OX-19 mAb, supporting the conclusion that T cells are not required for the LPS-induced recurrence of arthritis in this model. Several other mAbs recognizing other rat leukocyte antigens were also tested. Differences observed in the splenocyte profiles include a decreased percentage of W3/25+ cells and an increased percentage of MRC OX-6+ and MRC OX-12+ cells in rnu/rnu rats. These differences have been noted recently by Vaessen, et al. (36) in a comparison of WAG rnu/rnu and rnu/+ rats of the same age (2 mo) as those used in the present study. In addition, we noted an increased percentage of MRC OX-42+ cells in rnu/rnu rats.

Effect of In Vivo Administration of Mutanolysin on LPS-induced Recurrence of
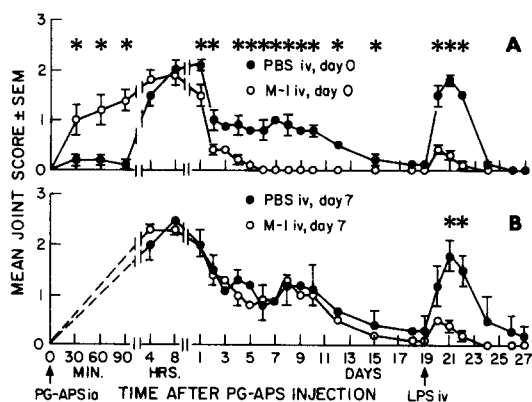
FIGURE 4. Clinical course of arthritis induced in Lewis rats showing the protective effect of mutanolysin treatment. On day 0, the right ankle joint of each of 19 Lewis rats was injected intraarticularly with 5 μg of PG-APS. Left ankle joints received PBS (not shown). (A) Immediately after PG-APS injection, six rats were injected intravenously with 400 μg of mutanolysin (O), and five rats received PBS (●). (B) On day 7, five rats were injected intravenously with 400 μg of mutanolysin (O), and three rats received PBS (●). On day 19, all rats were injected intravenously with 25 μg of S. typhimurium LT-2 LPS. Asterisks indicate times at which the mean joint score for PBS-treated rats was significantly higher than that for mutanolysin-treated rats (t test for independent means, $p < 0.025$).

Arthritis. The intravenous injection of mutanolysin on day 0, immediately after the intraarticular injection of PG-APS, resulted in an edematous reaction in the ankle joints that was evident within 30 min (Fig. 4A). This reaction did not occur in contralateral PBS-injected joints, nor in rats treated with PBS intravenously immediately after intraarticular injections. By 4 h, arthritis in mutanolysin- and PBS-treated rats was of the same severity, but after 24 h, arthritis in mutanolysin-treated rats subsided more quickly than that in PBS-treated rats. Administration of mutanolysin on day 7 had no effect on the rate at which the residual arthritis subsided (Fig. 4B). The intravenous injection of LPS on day 19 induced a recurrence of arthritis in the PG-APS-injected joints of PBS-treated rats that was of higher incidence, severity, and duration than that seen in mutanolysin-treated rats (Fig. 4, A and B). Some inflammation was also noted in contralateral PBS-injected joints after the LPS injection, but no significant differences between mutanolysin- and PBS-treated rats were seen in these joints.

Discussion

This study demonstrates that the systemic injection of a single sublethal dose of LPS into rats induces a mild acute, transient arthritis in ~50% of previously uninjected rats. A similar transient acute arthritis in guinea pigs was described in 1957 by Jones and Carter (16). They used two or four daily injections of 0.5 mg i.v. of S. *paradysenteriae* type Z pyridine-extracted (37) somatic antigen. Acute transient polyarthritis after intraperitoneal injection of LPS into rats has also been reported (17a). Of greater significance is the finding that systemic injection of a relatively low dose (10 μg) of LPS induces a recurrence of arthritis in 100% of joints that have been previously inflamed by the intraarticular injection of PG-APS. The reaction is primarily a transient acute exudative process that is superimposed on the mild residual chronic synovitis induced by the initial intraarticular PG-APS injection. Thus, joint inflammation initiated by peptidoglycan polymers can be sustained by distinct bacterial cell wall structures derived

from indigenous as well as pathogenic bacteria. The timing of injections, the time of onset of inflammation, and the histological features of this model indicate that the recurrence of arthritis is not a Shwartzman reaction. In addition, a recurrence of arthritis cannot be induced by the systemic injection of LPS after an intraarticular injection of the homologous or a heterologous LPS.

LPS from a variety of organisms induces a recurrence of arthritis in this model. Three lines of evidence indicate that lipid A is the active moiety: (a) enterobacterial Re mutant LPS and an Re mutant-like gonococcal LPS are active; (b) polymyxin B, which binds to lipid A (38) and blocks many biological activities of LPS in vitro and in vivo (39, 40, reviewed in 41), blocks the arthropathic activity of Re mutant LPS; (c) E. coli lipid A is weakly active.

The different susceptibilities of inbred Lewis and Buffalo rats to LPS-induced recurrence of arthritis correlate with past studies showing that Lewis rats are more susceptible than Buffalo rats to arthritis induced by intraperitoneal (5, 42) or intraarticular (15) injection of PG-APS. This differential susceptibility was also noted after intraarticular LPS injection. Arthritis induced by intraperitoneal injection of PG-APS is under polygenic control (5, 42). Because local PG-APS injections were used in the present study, and because PG-APS can persist in both Lewis and Buffalo rat tissues (43), differences in susceptibility to arthritis in this model probably reflect genetic control of the inflammatory response to PG-APS and LPS, and not to differences in the distribution of these polymers to the joint.

Our finding that inflammation induced in nude (athymic) rats by intraarticular injection of PG-APS is less prolonged than that in euthymic rats is consistent with the less severe chronic arthritis seen in nude rats after a systemic PG-APS injection (44, 45). However, this did not influence the capacity of LPS to induce a recurrence of arthritis, indicating that T lymphocytes are not required for the transient acute reaction. A role for T lymphocytes in chronic synovitis after the acute reaction cannot be excluded by the present studies, because all rats were killed 3 d after LPS injection.

Phlogistic agents may exacerbate disease by localizing in previously injured tissue (46). Past studies of reactivation of arthritis induced by intravenous injection of homologous or heterologous ^{125}I-labeled PG-PS revealed that more PG-PS localizes to the joint previously injured by intraarticular injection of PG-APS than to the contralateral PBS-injected joint, presumably due in part to a prolonged increase in joint vascular permeability (7). A synergistic effect of LPS and muramyl dipeptide, the minimum essential peptidoglycan structure that possesses many of the activities of peptidoglycan (47), has been demonstrated in an in vivo model of antitumor activity (48) and in in vitro adjuvanticity assays (49). More recently, Galelli and Chedid (50) reported a remarkable synergistic effect of muramyl dipeptide and LPS on the induction of colony-stimulating activity in mice.

We propose that the LPS-induced reactivation of arthritis described here results from a complementary or synergistic interaction of the phlogistic activities of LPS and PG-APS. Such an interaction would likely involve the shared biologic properties of these polymers (2) and may be related to structural similarities in the lipid A and muramyl dipeptide moieties (51). LPS may play a particularly

important role in the link between reactive arthritis and gastroenteritis caused by gram negative enteric pathogens such as *Y. enterocolitica* (52) and *S. typhimurium* (53); as well as in aseptic arthritis associated with disseminated gonococcal infection (54).

Summary

Rat ankle joints injected intraarticularly with 5 μg of group A streptococcal peptidoglycan-polysaccharide (PG-APS) developed an acute course of arthritis. Recurrence of arthritis was induced in 100% of these joints by intravenous injection of as little as 10 μg of *Salmonella typhimurium* lipopolysaccharide (LPS) 3 wk after intraarticular injection. This reaction was similar in athymic and euthymic rats. Buffalo rats were less susceptible than Lewis or Sprague-Dawley rats. *Neisseria gonorrhoeae*, *Yersinia enterocolitica*, and *Escherichia coli* LPS, and *S. typhimurium* Re mutant LPS, were also active. Re mutant LPS activity was greatly reduced by mixing with polymyxin B. *E. coli* lipid A was weakly active. An acute synovitis of much less incidence, severity, and duration was seen in contralateral joints injected initially with saline, and in ankle joints of naive, previously uninjected rats after intravenous LPS injection. The intravenous injection of the muramidase mutanolysin on day 0 or 7 after intraarticular PG-APS injection prevented LPS-induced recurrence of arthritis. These studies suggest that the phlogistic activities of lipid A and peptidoglycan might interact in an inflammatory disease process, and that LPS may play a role in recurrent episodes of rheumatoid arthritis or reactive arthritis.

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