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Chemical and Biological Characterization of the Lipopolysaccharide of the Oral Pathogen *Wolinella recta* ATCC 33238

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To investigate the potential pathogenic mechanisms of the oral periodontopathogen *Wolinella recta* ATCC 33238, we have isolated its lipopolysaccharide (LPS) and determined the chemical composition and selected in vitro biological activities of the molecule. Sodium desoxycholate-polyacrylamide gel electrophoresis revealed the *W. recta* LPS to be an atypical smooth LPS with short O-antigenic side chains. Chemically the LPS consisted of 47.2% lipid A, 19.6% polysaccharide, 9.0% heptose, 8.5% hexosamine, 3.2% phosphate, and 0.6% 2-keto-3-deoxyoctanoate. The major fatty acids were hexadecanoic acid (25.0%), 3-OH tetradecanoic acid (23.8%), tetradecanoic acid (15.4%), 3-OH hexadecanoic acid (11.6%), and octadecenoic acid (10.9%). Rhamnose constituted 87.8% of the carbohydrates generally associated with the O antigen, with smaller amounts of glucose (5.5%), mannose (4.9%), and an unidentified sugar (1.9%). CD-1 and C3H/HeN macrophages (Mϕ) exposed to 1 μg of *W. recta* LPS per ml released 6.0 and 10.5 ng of prostaglandin E per ml of supernatant, representing 625% and 1,306% of prostaglandin E release by the control (without LPS). Maximum prostaglandin E release occurred in CD-1 Mϕ exposed to 100 μg of LPS per ml and was equivalent to 1,542% of release by the control. Interleukin-1 (IL-1) activities in CD-1 and C3H/HeN Mϕ exposed to 1 μg of LPS per ml were 257% and 1,941% of activities in the control, respectively. Maximum IL-1 release in CD-1 Mϕ occurred in response to 50 μg of LPS per ml and represented a 927% increase over release in the control, while 100 μg of LPS per ml stimulated maximum IL-1 release in C3H/HeN Mϕ that was >5,000% of release by the control.

Members of the genus *Wolinella* are gram-negative, anaerobic, oxidase-positive, motile rods that grow best in complex media containing formate and fumarate (12, 43). *Wolinella* spp. have been implicated in periodontal disease, alveolar bone abscesses (43), root canal infections (14), and Crohn's disease (45); however, the direct role of this genus and that of their cell-associated components in the etiology of these diseases are unclear.

Many of the cell components which have been shown to function as virulence factors in gram-negative bacteria are associated with the bacterial surface. Of these, lipopolysaccharide (LPS) has been characterized as one that mediates a number of biological activities which can lead to the destruction of host tissues (36). Many of the detrimental effects of the LPS molecule result from endogenous mediators that are overproduced by host cells in response to challenge with LPS. These include tumor necrosis factor, endogenous pyrogen, interferon, glucocorticoid antagonizing factor, plasminogen activator, histamine, and serotonin (36). LPS also induces arachidonic acid metabolism with subsequent platelet aggregation (22) and prostaglandin E (PGE) production (29), stimulates macrophages (Mϕ) and monocytes to produce interleukin 1 (IL-1) (9), and elicits proteinases, including proteoglycanase and collagenase, from Mϕ and fibroblasts (16).

LPS has been implicated as a virulence factor in bacteria associated with periodontal disease, because it is known to penetrate gingival epithelial tissue (38) and stimulate bone resorptive activity (32). To investigate the virulence potential of the oral *Wolinella* sp. *Wolinella recta*, as well as its possible contribution to periodontal disease, we have isolated the LPS from *W. recta* ATCC 33238 and characterized its chemical and biological properties.

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MATERIALS AND METHODS

Culture. *W. recta* ATCC 33238, originally isolated from a periodontitis patient (43), was used in this study. The culture was maintained on enriched Trypticase soy agar plates (BBL Microbiology Systems, Cockeysville, Md.) and in mycoplasma-formate-fumarate broth (12). LPS was isolated from batch cultures of *W. recta* grown in mycoplasma-formate-fumarate broth.

LPS isolation. The cold $MgCl_2$-ethanol precipitation procedure described by Darveau and Hancock (8) was used to extract the LPS.

Analytical methods. The analytical methods listed in Table 1 were used.

Lipid A hydrolysis. LPS was hydrolyzed with 1% (vol/vol) acetic acid to yield lipid A and polysaccharide fractions as described by Kiley and Holt (22), except that the final drying step was at room temperature. The percentage of lipid A was determined gravimetrically.

Fatty acid analysis. Fatty acid methyl esters of lipid A and intact LPS were prepared by a variation of the method of Kropinski et al. (25). After hydrolysis of 1- to 3-mg samples of LPS at 100°C in 2 M methanolic HCl, the solvent was removed by a stream of nitrogen and the residue was suspended in 0.5 ml of *n*-hexane. A 1- to 2-μl portion of the *n*-hexane solution was analyzed by gas chromatography (GC) in a Schimadzu GC-9A gas chromatograph interfaced to a Schimadzu Chromatopac C-R3A printer (see Table 3 for analysis parameters).

Carbohydrate analysis. Intact LPS, lipid A, and polysaccharide were hydrolyzed as described by Kiley and Holt (22) and then converted to their alditol acetate derivatives by the

TABLE 1. Colorimetric methods for analysis of the *W. recta* ATCC 33238 LPS

Component	Method type or source	Standard	Reference
Total carbohydrate	Phenol sulfuric acid	Glucose	10
Neutral carbohydrate	Anthrone	Galactose	42
Heptose	Cysteine-sulfuric acid	Sedoheptulose	48
Hexosamine	Augustyniak and Augustyniak	Glucosamine	2
Total phosphate	Ames	Sigma phosphate standard	1
KDO	Karkhanis	KDO	21
	Brade et al.	KDO	5
	Caroff et al.	KDO	7
Nucleic acids	UV $A_{260-280}$		
Protein	Lowry	BSA[a]	30

[a] BSA, Bovine serum albumin.

procedure of Hill et al. (17); however, the samples were dried under nitrogen at 48°C, and the amino sugars were reduced at 0°C (27) (see Table 3, footnote b, for methods of alditol acetate analysis by GC).

GC-mass spectrometry. Electron impact (70 eV) mass spectra of the fatty acid methyl esters and alditol acetates were obtained on a Finnigan-MAT 212 mass spectrometer in combination with an INCOS 2200 data system. The ion source temperature was 250°C, and the accelerating voltage was 3 kV. Samples were introduced into the mass spectrometer from a Varian 3700 gas chromatograph which was directly connected via a heated transfer region.

Gel filtration. The molecular size of aggregated LPS was determined by elution through Sepharose 2B and 4B gel columns (Pharmacia Fine Chemicals, Uppsala, Sweden) (22).

Electrophoresis. Polyacrylamide gel electrophoresis (PAGE) was performed on vertical slab gels containing either sodium dodecyl sulfate (SDS) or sodium desoxycholate (DOC). SDS-PAGE used the Laemmli buffer system (26) with either 10 or 12.5% (wt/vol) acrylamide gels containing 0.2% (wt/vol) SDS. The SDS-PAGE system of Kropinski et al. (25) used 2% (wt/vol) SDS and 0.9 mM EDTA in the 10% (wt/vol) acrylamide separating gel and 2% (wt/vol) SDS and 2 mM EDTA in the 4.5% (wt/vol) acrylamide stacking gel and in the sample buffer. DOC-PAGE was carried out in 12.5% (wt/vol) acrylamide gels (4). LPS bands in the SDS-PAGE gels were visualized by the Hitchcock and Brown (18) modification of the Tsai and Frasch silver stain. DOC-PAGE gels were stained by the method of Tsai and Frasch (44).

In vitro biological assays. The effects of the *W. recta* LPS on the activation and viability of human peripheral monocytes (HPM) and murine Mϕ were studied by measuring the activities of extracellular and Mϕ-associated (intracellular) acid phosphatase (AcP) and extracellular lactate dehydrogenase (LDH). Cell viability was determined by trypan blue exclusion. Resident peritoneal Mϕ were obtained from CD-1, C3H/HeN, and C3H/HeJ mice by peritoneal lavage (3); HPM were obtained from healthy volunteers.

Cultivation of HPM and Mϕ as well as assays for AcP and trypan blue exclusion were as described by Barker and Holt (3). In this study, both cell types were used at concentrations of 5×10^5 cells per ml. Trypan blue exclusion by viable Mϕ was calculated as the percent viable control cells ± the standard error of the mean. AcP activity was calculated as the amount of *p*-nitrophenyl (in nanomoles) produced per 0.5 h per 5×10^5 Mϕ; LDH activity was assayed by the diphenylhydrazine procedure described by Cabaud and Wroblewski (6). Both AcP activity and LDH units are expressed as percent control-cell enzyme levels. Enzyme standards and reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.).

LPS stimulation of Mϕ. The LPS used in the bioassays was suspended at 20× its final concentration in sterile phosphate-buffered saline and sonicated by a Kontes microultrasonic cell disruptor for approximately 30 s to assure complete suspension of the LPS. It was then diluted in Dulbecco modified Eagle medium (DMEM), and at zero time, 1 ml of the same medium containing 1, 5, 50, or 100 μg of LPS was added to 5×10^5 Mϕ or HPM. Before the addition of LPS, the cell population was washed three times with serum-free DMEM. Medium without LPS served as a negative control.

PGE and IL-1 assays. Mϕ were incubated in DMEM at 37°C in 5% CO_2 with either 0, 1, 5, 50, or 100 μg of LPS per ml. In order to ensure against interference with IL-1 production by PGE, the cyclooxygenase inhibitor indomethacin (Sigma Chemical Co.) was added to the IL-1 assays at a final concentration of 0.02 mM. For PGE assays, samples were in indomethacin-free media with the exception of control Mϕ cultures, which were incubated with 50 μg of LPS plus 0.02 mM indomethacin, thus ensuring that the PGE we detected resulted from de novo synthesis. *Escherichia coli* 0127:B8 LPS (Sigma Chemical Co.) served as a positive control; negative controls did not contain LPS.

Supernatants from the LPS-stimulated Mϕ were collected at 24-h intervals for up to 6 days and were assayed for PGE by radioimmunoassay (Seragen, Boston, Mass.). IL-1 activity was determined by the thymocyte proliferation assay of Mizel (31). PGE levels are reported as nanograms of PGE per milliliter of Mϕ culture supernatant +/− the standard error of the mean, and IL-1 activities are expressed as counts per minute +/− the standard error of the mean. Significance testing of IL-1 and PGE data used the Student *t* test.

RESULTS

Chemical composition of LPS. The chemical composition of the *W. recta* LPS is shown in Table 2. Lipid A was the most abundant constituent of the LPS, with smaller amounts of polysaccharide, heptose, hexosamine, phosphate, and 2-keto-3-deoxyoctanoate (KDO) present as well. Protein contamination varied between 0.1 and 3% depending on the preparation; however, PAGE analysis of 100 μg of LPS did not reveal any protein bands after Coomassie brilliant blue staining. Nucleic acids were not detected.

GC-mass spectrometry analysis of alditol acetates derived from intact LPS (Fig. 1A) and from the polysaccharide fraction (data not shown) of *W. recta* indicated that rhamnose, glucose, mannose, and an unidentified sugar were

TABLE 2. Chemical composition of the *W. recta* ATCC 33238 LPS

Component	% Dry wt[a]
Total carbohydrate	19.6
Neutral carbohydrate	13.6
Heptose	9.0
Hexosamine	8.5
Total phosphate	3.2
KDO	0.6[b]
Nucleic acids	ND[c]
Protein	0.1–3.0
Lipid A	47.2

[a] Results are the averages of at least two determinations.
[b] Average of Karkhanis, Caroff, and Brade assays.
[c] ND, Not detected.

present, in a ratio of 8.8:0.6:0.5:0.2. The unidentified sugar is possibly a dideoxyhexose, since its GC retention time and mass spectrum are similar, but not identical, to those of abequose. Quantitative analysis by GC (Table 3) revealed that rhamnose constituted 87.8% of the sugars generally associated with the O antigen. In addition to the four sugars described above, two more alditol acetate peaks with retention times of 20.3 and 22.9 min (Fig. 1B) were found. The compound which eluted at 22.9 min eluted with the same retention time as the alditol acetate derived from the heptose of *Salmonella typhimurium*. Moreover, the mass spectra of the two *W. recta* peaks were essentially identical not only to the heptose in *S. typhimurium* but also to the published spectrum of L-glycero-D-mannoheptose (46), thus confirming the identity of these *W. recta* components as heptoses. It was, furthermore, found that the lipid A fraction contained only glucosamine. The derivative which provided the spectrum shown in Fig. 2 had the same GC retention time and mass spectrum as an authentic standard of glucosamine-hexaacetate.

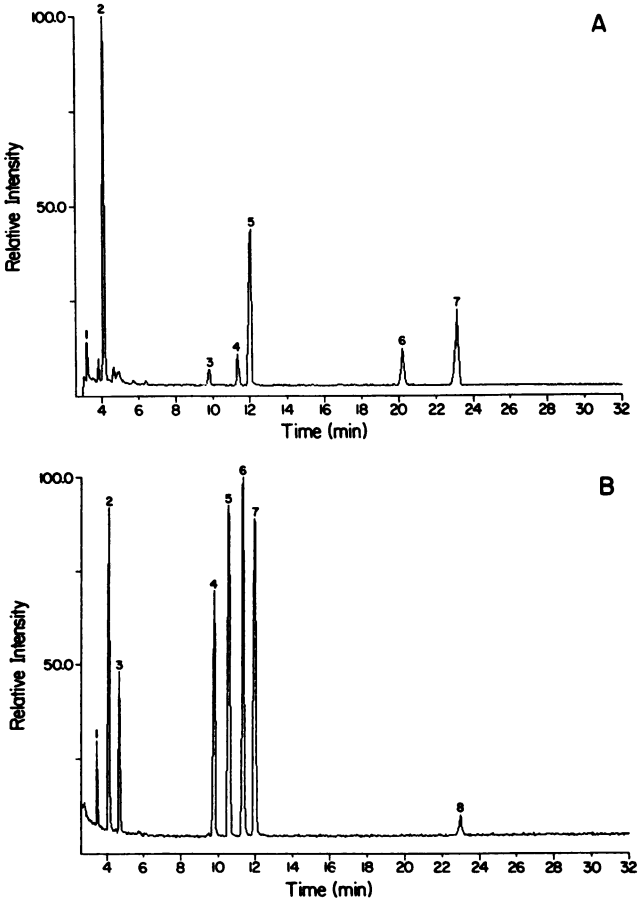

FIG. 1. Reconstructed ion chromatograph from GC-mass spectrometry analysis of alditol acetates derived from LPS of *W. recta* (A) and *S. typhimurium* (B). The analyses were performed as described in Materials and Methods by using the following GC conditions: injector temperature, 220°C; splitter flow, 20 ml/min; linear velocity of helium, 40 cm/s; injection volume, 1 μl; column temperature, 220°C; column, 30 m by 0.25 mm DB225, 0.25-μm film thickness (J & W Scientific). Peak identities for peracetates in panel A are as follows: 1, presumed alditol acetate (not identified); 2, rhamnitol; 3, mannitol; 4, glucitol; 5, inositol (internal standard); 6 and 7, presumed heptitols. For panel B, the assignments of the peracetates are as follows: 1, abequitol; 2, rhamnitol; 3, ribitol; 4, mannitol; 5, galactitol; 6, glucitol; 7, inositol (internal standard); 8, heptitol.

TABLE 3. Neutral monosaccharide and fatty acid compositions of *W. recta* ATCC 33238 LPS

Substance	% of total	nmol/mg of LPS[a]
Monosaccharide[b]		
Rhamnose	87.8	908.6
Glucose	5.5	51.5
Mannose	4.9	46.5
Unidentified	1.9	21.0
Fatty acid[c]		
Dodecanoic ($C_{12:0}$)	6.6	72.8
Tetradecanoic ($C_{14:0}$)	15.4	147.8
3-OH tetradecanoic (3-OH-$C_{14:0}$)	23.8	213.4
Hexadecanoic ($C_{16:0}$)	25.0	214.8
3-OH hexadecanoic (3-OH-$C_{16:0}$)	11.6	92.4
Octadecanoic ($C_{18:1}$)	10.9	84.7
Octadecanoic ($C_{18:0}$)	6.6	50.7

[a] Results are the averages of at least four determinations.
[b] GC conditions for analysis of monosaccharides (analyzed as alditol acetates) were as follows: 3% SP-2340 on 100/120 Supelcoport (Supelco) glass column (inside diameter, 2.5 m by 3 mm); injector and detector temperature, 250°C; column temperature, 225°C; carrier gas, nitrogen (flow rate, 40 ml/min). Weight percent determinations were made by using a myoinositol internal standard. To calculate the concentration of the unidentified carbohydrate, a molecular weight of 150 was assumed.
[c] GC conditions for analysis of fatty acids (analyzed as fatty acid methyl esters) were as follows: 3% SP-2100 DOH on 100/120 Supelcoport (Supelco) glass column (inside diameter, 2.5 m by 3 mm); injector temperature, 200°C; detector temperature, 250°C; program, 150°C for 4 min to 230°C at 4°C/min; carrier gas, nitrogen (flow rate, 20 ml/min). Weight percent determinations were made by using a methyl nonadecanoate internal standard.

The distribution of fatty acids in the *W. recta* lipid A is shown in Table 3. Seven fatty acids were identified. Hexadecanoic, 3-OH tetradecanoic, and tetradecanoic acids were predominant, with lower proportions of 3-OH hexadecanoic, octadecenoic, dodecanoic, and octadecanoic acids present as well.

Gel filtration chromatography. The aggregated molecular weight of *W. recta* LPS as determined by Sepharose 4B and 2B column chromatography was greater than two million (data not shown). On Sepharose 4B, the LPS eluted at a volume of 2.3× the void volume, while on Sepharose 2B it eluted in two peaks, one at 2.3× and the other at 1.2× the void volume. It is possible that aggregates of at least two molecular weight species were present.

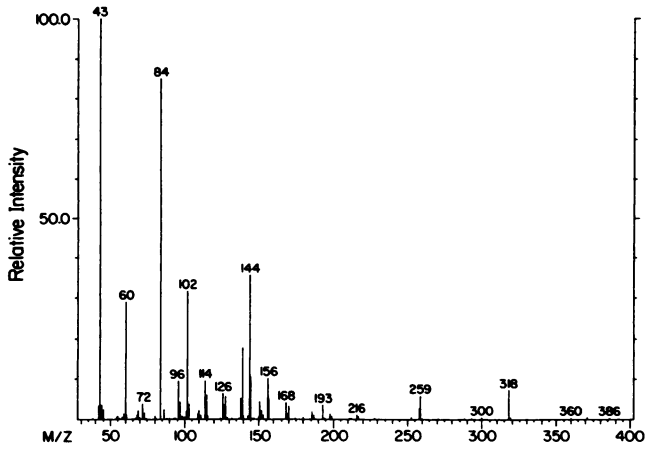

FIG. 2. Electron impact mass spectrum of glucosaminitol hexaacetate derived from the lipid A fraction of *W. recta*. Mass spectrometric conditions are described in Materials and Methods. Gas chromatographic separation was effected on a DB1701 fused silica capillary column (30 m by 0.25 mm) with a film thickness of 0.25 μm (J & W Scientific), by using helium at a linear velocity of 40 cm/s. The GC conditions were as follows: injector temperature, 250°C; splitter flow, 20 ml/min; injection volume, 6 μl; column temperature program, 200°C held for 1 min followed by an increase of 10°C/min to 260°C. Background contributions have been subtracted from the spectrum.

PAGE profiles. Figure 3 shows the analysis of LPS by SDS-PAGE and DOC-PAGE. When run on 12.5% acrylamide gels plus 0.2% SDS (Fig. 3A, lanes 1 and 2), *W. recta* LPS displayed a core band with an apparent molecular size of 13 kilodaltons (kDa), a singlet band at 18.5 kDa, and quadruplet bands at 26.6, 36.5, 55, 68, and 95.5 kDa. Four LPS bands also occurred between 116 and 180 kDa. When analyzed by DOC-PAGE (Fig. 3B, lanes 2 and 3) and by 2% SDS-PAGE plus EDTA (data not shown), the *W. recta* LPS displayed only the 13- and 26.6-kDa bands. The PAGE profile of the smooth *S. typhimurium* LPS was the same on both DOC-PAGE (Fig. 3B, lane 1) and SDS-PAGE (Fig. 3A, lane 3).

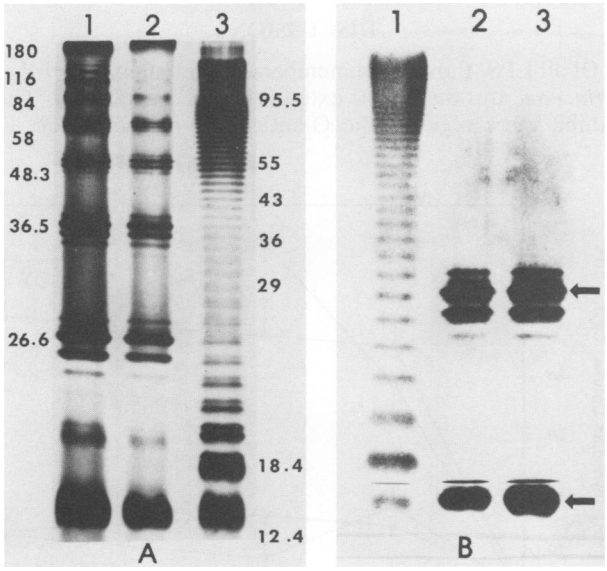

FIG. 3. (A) SDS-PAGE profiles of LPS on 12.5% acrylamide gels. Lanes: 1 and 2, *W. recta* at 10 and 5 μg; 3, *S. typhimurium* at 5 μg. Numbers indicate molecular weights (10^3) of Sigma molecular weight standards (left) and Diversified Biotech molecular weight standards (right). (B) DOC-PAGE profiles of LPS on 12.5% acrylamide gels. Lanes: 1, *S. typhimurium* at 5 μg; 2 and 3, *W. recta* at 5 and 10 μg. Arrows indicate positions of the 26.6- and 13-kDa bands.

Biological activity. The effects of the *W. recta* LPS on HPM and on resident Mϕ from outbred CD-1, LPS-responder C3H/HeN, and nonresponder C3H/HeJ mice were examined.

(i) Viability. When C3H/HeN and C3H/HeJ Mϕ and HPM were exposed to 100 μg of the *W. recta* LPS per ml for up to 144 h, viabilities (as determined by trypan blue exclusion) were essentially 100% of control levels and LDH was not detected in culture supernatants. In CD-1 Mϕ, the viability decreased to 90% at 144 h. Accompanying this decline in CD-1 Mϕ viability was an increase in extracellular LDH activity from 100% of activity in control at 48 h to 160% of activity in control at 144 h (data not shown).

(ii) AcP. Both HPM and CD-1 Mϕ exposed to *W. recta* LPS were assayed for intracellular accumulation as well as excretion of the lysosomal enzyme AcP. Exposure of HPM to 100 μg of LPS per ml for 24 h resulted in an intracellular concentration of AcP which was equivalent to 157% of AcP concentration in the control. Extracellular AcP was not detectable at 24 h; however, at 48 h, extracellular AcP rose slightly to 128% of the level in the control, while the intracellular AcP activity declined to 91% of the level in the control. This increase in extracellular AcP was not accompanied by loss of viability or elevations in LDH activity and therefore was not due to cell lysis. These observations are consistent with LPS stimulation of the selective release of HPM lysosomal enzyme.

When CD-1 Mϕ were exposed to 100 μg of LPS per ml for 24 h, the intracellular AcP activity rose to a level which was equal to 121% of activity in the control and remained constant for an additional 120 h. Extracellular AcP was not detected for 48 h; however, at 144 h, extracellular AcP was at 222% of the level in the control. This increase in extracellular AcP was accompanied by loss of viability and increased extracellular LDH activity (see above) and was most likely due to cell lysis rather than selective enzyme excretion.

(iii) LPS stimulation of PGE release. The 24-h PGE responses elicited by various concentrations of *W. recta* and *E. coli* LPS are shown in Fig. 4. At all concentrations tested, the *W. recta* LPS stimulated significant PGE release. The C3H/HeN Mϕ produced essentially equivalent PGE levels at LPS concentrations between 1 and 100 μg/ml. In the CD-1 Mϕ, the PGE response was depressed at 1 μg of LPS per ml, but at LPS concentrations of 5 to 100 μg/ml, there was an apparent saturation of PGE production. Compared with levels in control CD-1 and C3H/HeN Mϕ, PGE levels, in response to 1 μg of LPS per ml, increased by 625 and 1,306%, respectively. Maximum PGE levels in these Mϕ occurred in the presence of 100 and 50 μg of LPS per ml and represented 1,542 and 1,438% of levels in the control. Mϕ from the LPS-nonresponder C3H/HeJ mice did not produce PGE when challenged with *W. recta* LPS (data not shown).

The *E. coli* LPS also stimulated the release of high concentrations of PGE (Fig. 4). When CD-1 Mϕ were exposed to 1 μg of *E. coli* LPS per ml for 24 h, PGE concentrations increased by 740%. Maximum PGE levels occurred in the presence of 100 μg of LPS per ml and represented 1,411% of levels in the control.

The time course of PGE release in the presence of 50 μg of

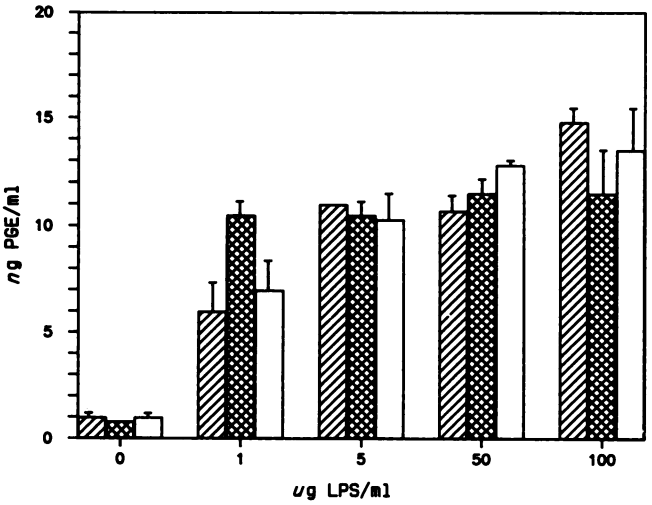

FIG. 4. Effects of various concentrations of LPS on PGE release by resident murine Mϕ. *W. recta* LPS with CD-1 (▨) and C3H/HeN (▩) and *E. coli* LPS with CD-1 (▭). Mϕ were exposed to LPS for 24 h. If no error bars are shown, the standard error of the mean was less than 0.05.

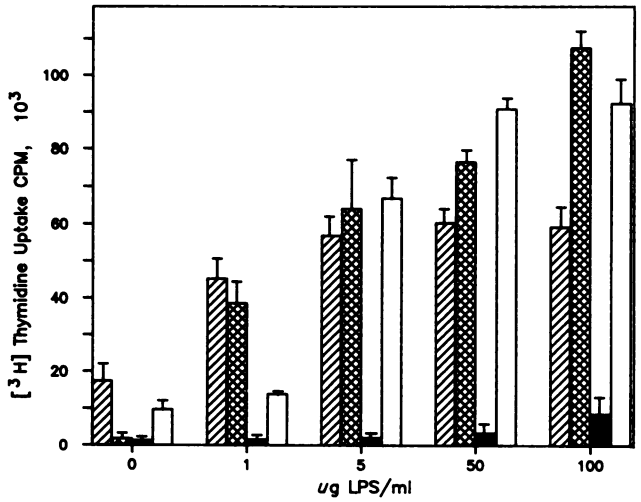

FIG. 6. Effects of various concentrations of LPS on IL-1 release by resident peritoneal Mϕ. *W. recta* LPS with CD-1 (▨) C3H/HeN (▩), and C3H/HeJ (■) and *E. coli* LPS with CD-1 (▭). Mϕ were exposed to LPS for 24 h; supernatants were diluted one-fourth.

LPS per ml is shown in Fig. 5. There was an initial rapid rise to maximum PGE release in the first 24 h of the experiment, and there was no significant decline in PGE activity for the remaining 120 h. The time course of PGE production by LPS-stimulated Mϕ did not vary with either the source or the dosage of LPS (data not shown).

(iv) LPS stimulation of IL-1 release. We have examined the elicitation of IL-1 from murine Mϕ exposed to LPS from *W. recta* and *E. coli* (Fig. 6). At 24 h, all concentrations tested (1 to 100 μg of LPS per ml) stimulated a significant increase in IL-1 production in both CD-1 and C3H/HeN Mϕ. In these Mϕ, *W. recta* LPS at a concentration of 1 μg/ml elicited increases in IL-1 activities to 257 and 1,941% of the activity in the control. In CD-1 Mϕ, the maximum response to the *W. recta* LPS occurred at 50 μg/ml, with IL-1 levels increasing to 927%. The C3H/HeN Mϕ required exposure to 100 μg of LPS per ml to produce maximum IL-1 activity, 5,375% of activity in the control. IL-1 activity in response to 1 and 100 μg of *E. coli* LPS per ml was 142 and 727% of activity in the control; the latter was the maximum response. The levels of IL-1 produced in LPS nonresponder C3H/HeJ mice were not significantly elevated over the levels in the control.

The time course of IL-1 activity in response to 1 μg of LPS per ml is seen in Fig. 7. A concentration of *W. recta* LPS of 1 μg/ml stimulated significantly higher ($P < 0.002$) IL-1 activity than did 1 μg of *E. coli* LPS per ml, and this significant difference was sustained for the duration of the experiment.

DISCUSSION

Of all LPS, those from members of the family *Enterobacteriaceae* are the most extensively characterized. They exhibit three regions: the O-antigenic polysaccharide, the

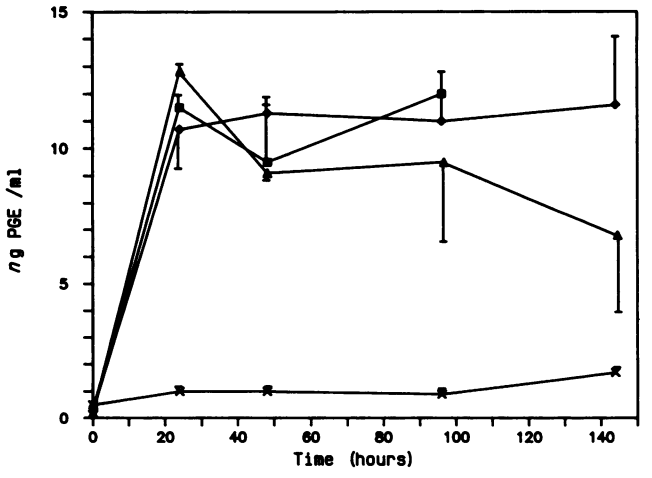

FIG. 5. Time course of PGE release by LPS-stimulated resident peritoneal Mϕ. Fifty micrograms of either *W. recta* LPS with CD-1 (◆) and C3H/HeN (■) or *E. coli* LPS with CD-1 (▲) and control (no LPS) (×).

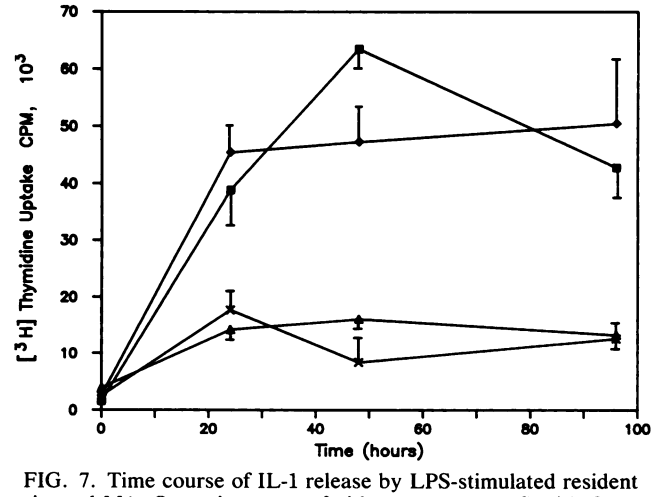

FIG. 7. Time course of IL-1 release by LPS-stimulated resident peritoneal Mϕ. One microgram of either *W. recta* LPS with CD-1 (◆) and C3H/HeN (■) or *E. coli* LPS with CD-1 (▲) and control (no LPS) (×). Mϕ supernatants were diluted one-fourth.

core oligosaccharide, and the lipid A (19). Because the enteric LPS is so well defined, it often serves as the prototype with which uncharacterized LPS is compared. So, for example, the LPS from the oral bacteria *Capnocytophaga sputigena* (33), *Eikenella corrodens* (34), *Actinobacillus actinomycetemcomitans* (22), *Bacteroides gingivalis* (23, 32), and *Bacteroides intermedius* (20) are chemically different from enteric LPS with regard to core oligosaccharide and fatty acid content. Specifically, they have lower levels of the core monosaccharide KDO, and the LPS from the *Bacteroides* spp. are devoid of heptose. Hydroxy-fatty acids have been detected only in *A. actinomycetemcomitans*, *B. intermedius*, and *B. gingivalis* (32), and β-OH myristate, common to the enteric LPS, occurs only in the *A. actinomycetemcomitans* LPS.

W. recta LPS differed from these other "oral" LPS in that it had all of the chemical constituents which have been reported in enteric LPS; however, compared with both enteric and oral LPS, it did have a significantly higher content of lipid A (47%) and a lower level of neutral carbohydrate (20%).

The polysaccharide moiety of the *W. recta* LPS was also of an unusual chemical composition, consisting of 88% rhamnose. The core-specific carbohydrates also appeared to include two molecular forms of heptose. Analogous O-antigen and core composition have been observed in LPS from plant bacteria (37, 40, 41). O antigens composed almost entirely of rhamnose were found in *Pseudomonas syringae*, *P. solanacearum*, and *P. cepacia* (40, 41). The core oligosaccharide of *Rhizobium trifolii* contains two methyl heptoses plus the common L-glycero-D-mannoheptose (37).

The *W. recta* LPS did have a low proportion of KDO and was like the other oral LPS in the paucity of this molecule. While KDO molecules that are heavily substituted by either heptose (5) or phosphate (5, 7) have been found to be refractory to detection in the thiobarbituric acid assay unless first subjected to either rigorous hydrolysis (5) or dephosphorylation (7), this was not the case for the *W. recta* LPS. In rough mutants of *Aeromonas salmonicida*, the existence of KDO in the furanose form is responsible for erroneously low KDO levels (39). Further research will be necessary to determine whether ring structure is a factor in the determination of KDO levels in *W. recta*.

The *W. recta* LPS cannot be classified as smooth or rough according to the criteria used for enteric LPS (19). Its low ratio of neutral sugar to lipid A (0.42) and its ability to form high-molecular-weight aggregates are typical of rough LPS (28). However, the four high-molecular-weight bands (approximately 26.6 kDa) in the *W. recta* LPS did comigrate with bands 5 through 8 of the *S. typhimurium* LPS, indicating the presence of O-antigenic side chains containing 5, 6, 7, and 8 repeating units. By definition, rough LPS does not possess O antigen, while semirough LPS has only one O antigen unit (19). Neither applies to *W. recta* LPS; it can best be characterized as an "atypical smooth LPS which contains five components with the smallest one equivalent to rough LPS" (44).

Because biological activities of LPS are associated with specific regions of the molecule (36), chemical analysis of undefined LPS can be a valuable means of predicting its role in bacterial virulence. The polysaccharide composition (i.e., 88% rhamnose, 5.5% glucose, 4.9% mannose, and 1.9% unidentified sugar) together with high β-OH fatty acid content in the *W. recta* LPS are chemical properties that could influence not only the interactions of LPS with mammalian cell surfaces but also its biological activity.

The pseudomonads and *Rhizobium* spp., with which *W. recta* LPS shares similar polysaccharide and core composition, offer good examples of how ligand-receptor interactions are affected by surface polysaccharide. For example, the bacteriophage infection of *P. syringae* requires that the virus possess a rhamnanase in order to cleave the rhamnan polymeric O antigen before it binds to its receptor site on the LPS core (40). Further, *R. trifolii*, which is routinely capable of infecting leguminous plants, cannot do so when excess heptose is incorporated into its LPS (37). It appears that the excess heptose interferes with binding to leguminous lectin and, therefore, renders *R. trifolii* unable to infect its plant host.

β-OH fatty acids constitute 35% of the fatty acids derived from *W. recta* lipid A, and 24% of this fatty acid moiety is β-OH myristic acid. Evidence supports the role of β-OH fatty acids in the endotoxic activity of enteric LPS (36), and in *E. coli* lipid X, an ester-linked β-OH myristoyl residue is responsible for activation of protein kinase C (47). Moreover, β-OH fatty acids are substrates for the formation of acyloxyacyl fatty acids, and a minimum number of properly placed acyloxyacyl groups are required for the biological activity of lipid A precursors (11) and synthetic lipid A analogs (24).

The *W. recta* LPS was capable of the in vitro activation of HPM to release alkaline phosphatase, as well as the elicitation of large amounts of IL-1 and PGE in murine Mϕ. The ability of periodontopathogenic bacteria to stimulate PGE and IL-1 production is of interest, because these immune factors mediate activities that could contribute to the pathology of periodontal disease. Both PGE and IL-1 are potent mediators of inflammation and bone resorption (9, 13, 29, 35), while IL-1 potentiates PGE production in monocytes, Mϕ, and fibroblasts and stimulates collagenase activity in fibroblasts (9).

In our experiments, even low doses of the *W. recta* LPS elicited levels of IL-1 that have been shown to stimulate significant bone resorptive activity. For example, purified IL-1 at a concentration with thymocyte proliferative activity of 21,000 cpm stimulated release of 31% of total bone isotope from ^{45}Ca-labeled mouse calvaria (13). A 1-μg portion of *W. recta* LPS stimulated approximately twice this level of IL-1 activity.

Little published information that compares the elicitation of PGE and IL-1 by LPS from periodontal pathogens is available. Hanazawa et al. (15) compared IL-1 activity in C3H/HeN Mϕ exposed to LPS from *B. gingivalis* and *E. coli*; at 10-μg doses, *E. coli* LPS was four times more active than the *B. gingivalis* LPS. We observed no significant differences between the levels of IL-1 elicited in response to 5 and 50 μg/ml of either *E. coli* or *W. recta* LPS. At 1 μg/ml, however, *W. recta* LPS elicited significantly more IL-1. Thus, by comparison with the results of Hanazawa et al. (15), we can conclude that *W. recta* LPS is capable of stimulating a stronger IL-1 response than that stimulated by the LPS from *B. gingivalis*. Recently, PGE_2 production in HPM exposed to LPS from *W. recta*, *B. gingivalis*, *B. intermedius*, *A. actinomycetemcomitans*, and *S. typhimurium* was compared (S. W. Garrison, S. C. Holt, and F. C. Nichols, J. Periodontol., in press). The *W. recta* LPS was the most active of those tested both in levels of PGE_2 elicited and in maintenance of the maximum response over the 72-h duration of the experiment. The levels of PGE elicited by *W. recta* LPS in our experiments were equivalent to those reported by Garrison et al., and we concur with their

observation that the LPS has the capacity to sustain a high PGE response over several days.

W. recta LPS then is chemically different from other oral LPS, and it may be a stronger elicitor of PGE and IL-1. Further research to determine how the chemical composition of *W. recta* LPS affects its biological activity is in progress. In light of the chemical distinctions among *W. recta*, enteric, and other oral LPS, this information may aid in elucidating how structural differences contribute to the biological potencies of various LPS preparations.

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