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Enzymatically Deacylated *Neisseria* Lipopolysaccharide (LPS) Inhibits Murine Splenocyte Mitogenesis Induced by LPS

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Acyloxyacyl hydrolase is a leukocyte enzyme that selectively removes the secondary acyl chains from the lipid A moiety of gram-negative bacterial lipopolysaccharides (LPS). As predicted by the reported contribution of secondary acyl chains to the bioactivities of lipid A analogs, enzymatic deacylation of *Salmonella typhimurium* Rc LPS substantially reduces its potency in the dermal Shwartzman reaction and in several in vitro assays that measure responses of human endothelial cells and neutrophils, whereas the potency of this LPS for inducing murine splenocyte mitogenesis is affected much less. In the experiments described here, we studied the impact of acyloxyacyl hydrolysis on the bioactivities of several LPS that differ from *Salmonella* LPS in carbohydrate and lipid A structures. Deacylated LPS from *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *S. typhimurium* were similarly reduced in potency in the *Limulus* lysate test (30- to 60-fold reduction in potency relative to the corresponding mock-treated LPS), and the ability of all of these deacylated LPS to stimulate neutrophil adherence to human endothelial cells was reduced by a factor of 100 or more. For LPS from *E. coli*, *H. influenzae*, and *Pseudomonas aeruginosa*, the impact of deacylation on spleen cell mitogenesis was also similar to that observed for *S. typhimurium* LPS: deacylation reduced potency by less than 15-fold. Unexpectedly, the potency of *Neisseria* LPS in the murine splenocyte mitogenicity test was reduced over 100-fold by deacylation, and deacylated *Neisseria* LPS could block the mitogenic activity of *Neisseria* and *Salmonella* LPS. These studies indicate that the contribution of secondary acyl chains to the bioactivities of a given LPS cannot be predicted with confidence from the reported structure-activity relationships of lipid A or from the behavior of other deacylated LPS.

The lipid A moiety of bacterial lipopolysaccharides (LPS) elicits virtually all of the responses of animals to LPS. Lipid A is a bisphosphorylated diglucosamine that is substituted by ester- and amide-linked 3-hydroxy acyl chains; some of the hydroxy acyl chains are themselves substituted with (secondary) acyl chains to form acyloxyacyl residues. Studies of chemically synthesized lipid A and its analogs have established that one or more secondary acyl chains are necessary for lipid A to elicit certain responses, including the dermal Shwartzman reaction, whereas analogs that lack these linkages retain many other lipid A bioactivities, including the ability to stimulate murine splenocyte mitogenesis (reviewed in reference 34).

To examine the contribution of the secondary acyl chains (or the acyloxyacyl groups) to the bioactivities of LPS, we treated *Salmonella typhimurium* Rc LPS with acyloxyacyl hydrolase (AOAH), an enzyme that selectively removes these acyl chains (Fig. 1A). The 3-hydroxy acyl chains that substitute the diglucosamine backbone are not released by the enzyme, and the carbohydrate chain of the LPS remains intact (13, 25). The lipid A moiety of enzymatically deacylated LPS thus resembles the lipid A analog known as compound 406 (lipid IV_A, precursor Ia, compound LA-14-PP [34]). Enzymatic deacylation of *S. typhimurium* LPS reduced its potency in the preparative phase of the dermal Shwartzman reaction by a factor of 100 or greater, while the ability of the deacylated LPS to stimulate murine splenocyte mitogenesis was reduced only 10- to 20-fold relative to the control LPS (25). These observations are consistent with the reported bioactivities of compound 406 (reviewed in reference 34) and suggest that, in these bioassays, the oligosaccharide chain and other properties of this LPS do not significantly modify the signal information contained in its acyloxyacyl linkages.

Although the general structure of LPS is highly conserved, LPS from different species differ in the nature and linkage of the acyl chains that substitute their lipid A moieties (3, 14, 15, 17, 31, 36, 37, 42; Fig. 1B) and in the structure of their carbohydrate chains; there is also microheterogeneity among LPS in the degree of phosphorylation of lipid A and of substitution with charged residues (31, 33). It was therefore of interest to examine the contribution of secondary acyl chains to the potency of LPS that differ from *S. typhimurium* LPS in lipid A and carbohydrate structures. We have recently found that AOAH carries out the same specific deacylation of such diverse LPS (6), and in the experiments described here we studied the impact of this structural alteration on certain bioactivities of these LPS. While the reduction in bioactivity for most of the LPS studied was similar to that found for deacylated *S. typhimurium* Rc LPS, we found that deacylated *Neisseria* LPS were surprisingly inactive in the murine splenocyte mitogenicity test and were even able to inhibit the mitogenicity of intact LPS.

MATERIALS AND METHODS

LPS. The cultivation of bacteria of various species and the preparation of their LPS have been described previously (6). LPS to be enzymatically deacylated were prepared from bacteria grown in media containing [^{3}H]acetate for intrinsic

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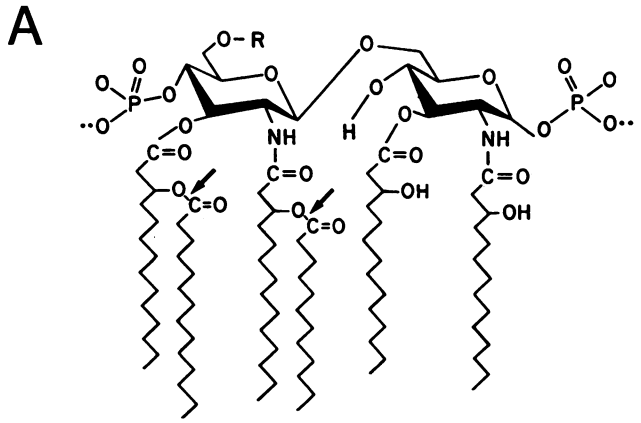

S. typhimurium [a]	3-O(14:0)-14:0	3-O(12:0)-14:0	3-OH-14:0	3-OH-14:0
E. coli [b]	3-O(14:0)-14:0	3-O(12:0)-14:0	3-OH-14:0	3-OH-14:0
H. influenzae [c]	3-O(14:0)-14:0	3-O(14:0)-14:0	3-OH-14:0	3-OH-14:0
N. gonorrhoeae [d]	3-OH-12:0	3-O(12:0)-14:0	3-OH-12:0	3-O(12:0)-14:0
P. aeruginosa [e]	3-OH-10:0	3-O(12:0)-12:0, 3-O(2-OH-12:0)-12:0	H	3-O(2-OH-12:0)-12:0, 3-O(12:0)-12:0

FIG. 1. Acyl substitution of LPS used in this study. (A) Lipid A moiety of *S. typhimurium* LPS (31, 37). The saccharide chain is attached to the nonreducing glucosamine, at R. Arrows indicate the sites at which AOAH catalyzes hydrolysis of the acyloxyacyl bonds. (B) Generalized structure for lipid A. The reported fatty acyl substitutions at positions R_2, R_3, $R_{2'}$, and $R_{3'}$ are indicated. The degree of acylation of LPS molecules is not uniform (31); with the exception of *P. aeruginosa*, the substitutions given are for the hexaacyl forms. [a] Reference 37; [b] reference 15; [c] reference 14; [d] reference 36. The lipid A structure of *N. meningitidis* has not been reported; the fatty acyl composition is the same as that reported for *N. gonorrhoeae* (17). [e] References 3 and 42; substitution at positions 2 and 2' may be with either of the acyloxyacyl groups listed (20a).

labeling of fatty acyl chains. Most bacterial isolates were the same as those used previously (6, 22); *Neisseria meningitidis* 6155 was obtained from Wendell D. Zollinger (Walter Reed Army Institute of Research, Washington, D.C.), and *Neisseria gonorrhoeae* 71H was obtained from Peter A. Rice (Boston University, Boston, Mass.). LPS were extracted with diethyl ether to remove contaminating phospholipids and were free of detectable nucleic acid contamination, as judged by their UV absorption spectra. None of the LPS preparations was mitogenic for splenocytes of C3H/HeJ (LPS-hyporesponsive) mice (data not shown).

Monoclonal antibodies. The preparation and characterization of mouse anti-LPS antibodies 3F11 (1, 22, 23), O6B4 (22, 23), 1-1-M (23), 6B7 (5), 2-1-L8 (23), 3G9 (23), and 1-9C4 (18) have been described. Antibodies were kindly provided by Michael A. Apicella (State University of New York, Buffalo; 3F11, O6B4, 1-1-M, and 6B7), Jan Poolman (National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands; 1-9C4), Peter A. Rice (3G9), and Wendell D. Zollinger (2-1-L8).

Enzymatic deacylation of LPS. Removal of secondary acyl chains by incubation with partially purified (at least 1,500-fold enriched [for *Haemophilus influenzae* LPS] and otherwise greater than 10,000-fold enriched) human AOAH was done as described previously (6, 25); enzyme-treated LPS and mock-treated LPS (subjected to the same incubation as for enzyme treatment, in buffer lacking enzyme) were suspended at 40 μg/ml in pyrogen-free saline and stored at −70°C. Preparations of enzyme-treated LPS and mock-treated LPS also contained bovine serum albumin (5 mg/ml) as well as small amounts of AOAH, as previously described (30). The specificity and completeness of deacylation were confirmed for each LPS by thin-layer chromatography of the fatty acids released or not released by enzymatic treatment (6).

Assays of LPS activity. (i) Adherence of leukocytes to endothelial cells was assayed as previously described (7, 27). Briefly, cultured human umbilical vein endothelial cells were incubated for 4 h with LPS and then washed and incubated with ^{51}Cr-labeled HL60 cells. After 30 min, nonadherent cells were removed, and the radioactivity associated with the endothelial cell monolayer was determined. Samples were assayed in quadruplicate; the standard errors for replicate wells were less than 20% of the mean ^{51}Cr counts per minute per well.

(ii) *Limulus* activity was determined by a chromogenic assay as previously described (7); samples were assayed in duplicate and the results were averaged.

(iii) Mitogenic activity of LPS for splenocytes of C3H/HeN mice was assayed by measuring the uptake of [^{3}H]thymidine as previously described (7, 25). Each sample was assayed in quadruplicate; the standard errors for replicate wells were less than 15% of the mean ^{3}H disintegrations per minute per well. For determination of viability and state of activation after incubation with LPS, splenocytes were incubated with 10 μM 6-carboxyfluorescein diacetate (Molecular Probes, Eugene, Oreg.) for 15 min at 37°C and then suspended in phosphate-buffered saline. Cell size was determined by forward-angle and side-scatter analysis, and fluorescence of living cells was determined by excitation with a 488-nm argon ion laser and emission at 530 nm (39), using a FACStar Plus (Becton Dickinson and Co., Rutherford, N.J.).

The differences in activity between enzyme-treated and mock-treated LPS were quantified graphically by determining the horizontal distance between their dose-response curves and calculating the ratios of the corresponding LPS concentrations. When possible, this determination was made at points on the linear regions of the curves corresponding to approximately 50% of the maximal stimulation seen in that assay. If the deacylated LPS did not approach half-maximal stimulation, the fold reduction reported was the ratio of the highest concentration of the deacylated LPS tested to the lowest concentration of the mock-treated LPS that exceeded the deacylated LPS in potency.

Antigenic characterization of *Neisseria* LPS. The binding of monoclonal antibodies to wells coated with LPS (untreated, enzyme-treated, or mock-treated LPS) was assayed in a solid-phase radioimmunoassay as previously described (23). Antibodies were diluted to a concentration that had been shown previously to result in saturation binding to a positive-control LPS.

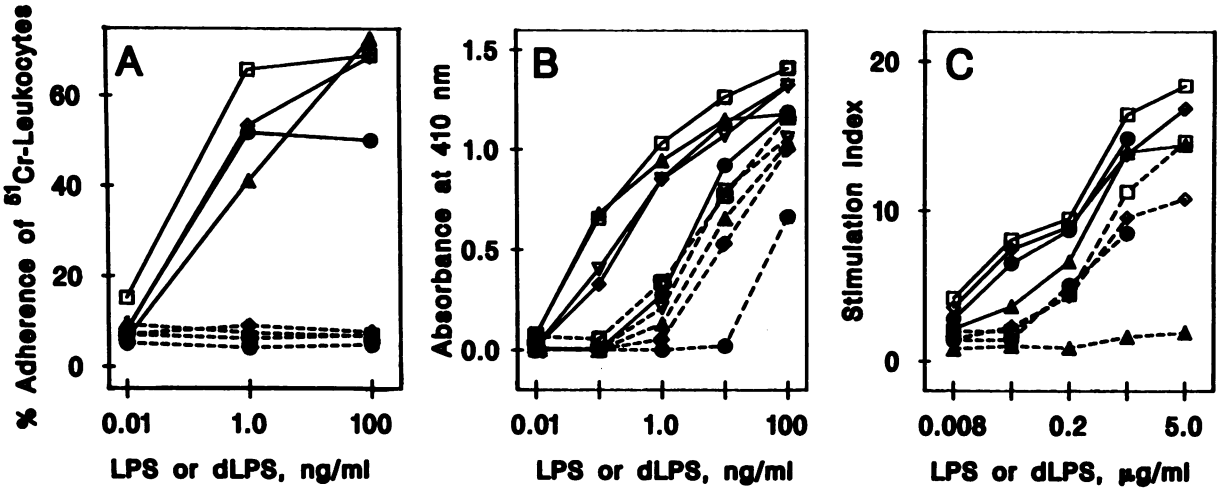

FIG. 2. In vitro activities of enzyme-treated LPS (dLPS, dashed lines) and mock-treated LPS (solid lines). (A) Adherence of ^{51}Cr-labeled HL60 cells to LPS- or dLPS-treated human umbilical vein endothelial cells. (B) *Limulus* activity, determined in a chromogenic assay. (C) Mitogenic activity for splenocytes of C3H/HeN mice. The stimulation index is the ratio of [^{3}H]thymidine incorporated into cells incubated with LPS or dLPS to [^{3}H]thymidine incorporated into control cells (4,169 ± 798 ^{3}H dpm per well [mean ± 1 standard deviation for four replicate wells]). ●, *S. typhimurium* PR122; ▲, *N. meningitidis* 8693; ▽, *N. meningitidis* 6155; □, *H. influenzae* DL42; ◇, *E. coli* J5.

RESULTS

We compared enzyme-treated LPS from three species of bacteria (*Escherichia coli*, *H. influenzae*, and *N. meningitidis*) with the corresponding mock-treated LPS in three in vitro assays of LPS activity (Fig. 2). As previously observed for *S. typhimurium* Rc LPS (27), the ability of these LPS to stimulate leukocyte-endothelial adherence was reduced over 100-fold by removal of the secondary acyl chains; the deacylated LPS had no detectable activity at the highest concentration tested (100 ng/ml) (Fig. 2A). In contrast, the same preparations of deacylated LPS had readily detectable *Limulus* activity at this concentration (Fig. 2B). Deacylation reduced *Limulus* activity 30- to 60-fold for the different LPS.

For LPS of *E. coli* and *H. influenzae*, the effect of deacylation on mitogenic activity for murine splenocytes was similar to that previously seen for *S. typhimurium* Rc LPS (25, 27): activity was reduced 9- to 13-fold (Fig. 2C). We also tested two smooth LPS (Fig. 3): for *Pseudomonas aeruginosa* K LPS, reduction was 12-fold (Fig. 3A); for LPS from *S. typhimurium* PR122 grown in the presence of galactose, mitogenic activity was reduced 6 fold by deacylation (Fig. 3B). Both of these LPS possessed long saccharide chains (confirmed by the characteristic ladder pattern [26] on silver-stained sodium dodecyl sulfate-polyacrylamide gels [data not shown]). In contrast, the effect of enzymatic deacylation on mitogenic activity of *N. meningitidis* LPS was much greater, over 100-fold reduction in potency (Fig. 2C).

In a number of systems, LPS or lipid A analogs that are inactive are able to inhibit the activity of normally active lipid A or LPS. We therefore tested deacylated meningococcal LPS to determine whether it would inhibit the mitogenic activity of intact LPS. We found that the deacylated LPS inhibited mitogenesis in a dose-dependent fashion when added to spleen cells along with mock-treated meningococcal LPS; a 10:1 ratio of deacylated LPS to mock-treated LPS resulted in maximal inhibition of mitogenic activity (Fig. 4). In a separate experiment, we determined that deacylated LPS was not overtly cytotoxic, as measured by uptake and cleavage of 6-carboxyfluorescein diacetate by cells har-

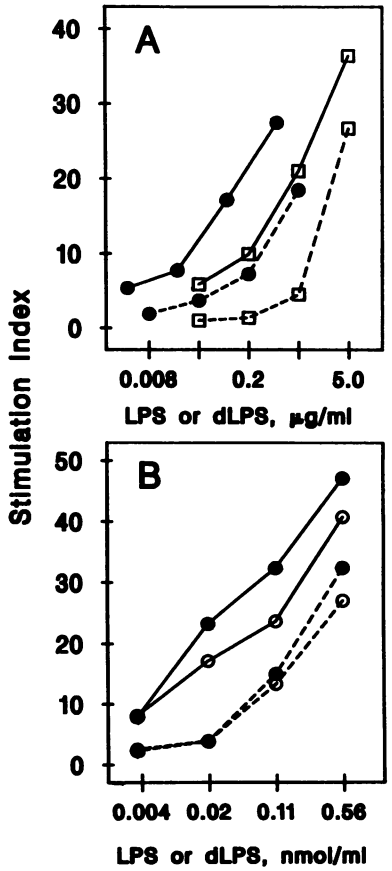

FIG. 3. Effect of enzymatic deacylation on splenocyte mitogenic activity of *P. aeruginosa* LPS (A, □) and smooth *S. typhimurium* LPS (B, ○), compared with Rc chemotype *S. typhimurium* LPS (●). Solid lines, mock-treated LPS; dashed lines, enzyme-treated LPS (dLPS). The molarity of the smooth *S. typhimurium* LPS was determined by assay of its glucosamine content (6). Control uptake for the assay in panel A was 3,178 ± 657 ^{3}H dpm per well; for the assay in panel B, it was 2,284 ± 243 ^{3}H dpm per well.

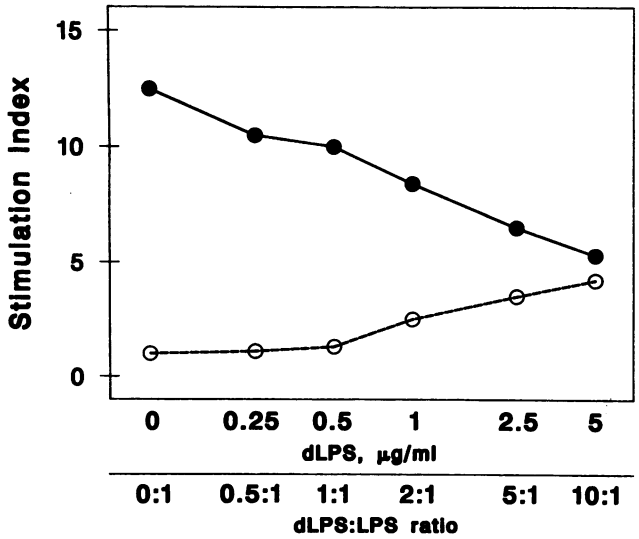

FIG. 4. Inhibition of splenocyte mitogenic activity of meningococcal LPS by enzymatically deacylated meningococcal LPS. Solid line, mitogenic activity of 0.5 μg of mock-treated LPS per ml incubated with splenocytes in the presence of added enzyme-treated LPS (dLPS) at the concentrations indicated on the *x* axis. Dashed line, mitogenic activity of enzyme-treated LPS alone. Incorporation into cells incubated without LPS was 3,089 ± 379 ^{3}H dpm per well.

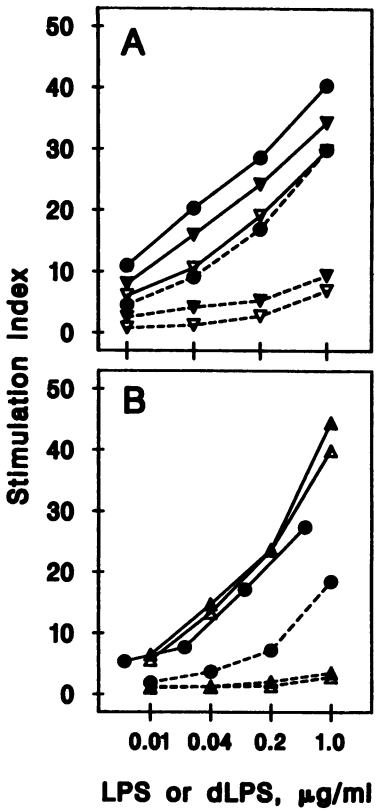

FIG. 5. Spleen cell mitogenicity of additional *Neisseria* LPS compared with *S. typhimurium* Rc LPS (●). (A) *N. meningitidis* isolates 7889 (▼) and 126E (▽). (B) *N. gonorrhoeae* isolates 24-1 (▲) and 71H (△). Solid lines, mock-treated LPS; dotted lines, enzyme-treated LPS (dLPS). Deacylation removed the mitogenic activity of all of the neisserial LPS to a greater degree than that of *S. typhimurium* Rc LPS. Similar results were seen for LPS of *N. meningitidis* 6155 (data not shown). The data for *S. typhimurium* Rc LPS in panel B are also shown in Fig. 2A. Control uptake for the assay in panel A was 3,178 ± 657 ^{3}H dpm per well; for the assay in panel B, it was 3,816 ± 1306 ^{3}H dpm per well.

vested after 48 h of incubation. Flow cytometry showed that there was no activation of B cells by the deacylated LPS and that the presence of deacylated LPS blocked activation by LPS, as assessed by blast formation.

Deacylated meningococcal LPS was also able to inhibit the mitogenic activity of *S. typhimurium* Rc LPS (data not shown). However, deacylated meningococcal LPS did not inhibit the splenocyte mitogenic response to concanavalin A (1 μg/ml; data not shown).

In order to determine whether these observations were limited to the particular isolate of *N. meningitidis* used (strain 8693), we prepared LPS from three additional isolates of *N. meningitidis* and from two isolates of *N. gonorrhoeae*. All of these LPS were greatly reduced in spleen cell-mitogenic activity after enzymatic deacylation (Fig. 5). The neisserial LPS differed in carbohydrate structure, as shown by their ability to bind several monoclonal antibodies reactive with the oligosaccharide region (Table 1; 18, 22). Quantitative analysis of antibody binding by enzyme-treated and mock-treated LPS from *N. gonorrhoeae* showed that while in some cases antibody binding appeared to be reduced by enzyme treatment, in no case was an epitope completely lost (Table 2).

DISCUSSION

Treatment of LPS with AOAH allowed us to examine the effect on bioactivity of one alteration of lipid A structure, the removal of secondary acyl chains, in the context of an otherwise intact LPS. We used LPS that differed in carbohydrate structure and in lipid A structure (Fig. 1B) and examined the effect of enzymatic deacylation of LPS in three in vitro assays of LPS bioactivity.

For neutrophil-endothelial cell adherence and for the *Limulus* assay, the results were similar for LPS of four different bacterial species, suggesting that the effect of the secondary acyl chains on potency in these assays was affected little by the structural variations among these LPS. These results were consistent with those seen with enzymatically deacylated *S. typhimurium* Rc LPS (27) and the synthetic lipid A analog 406 (34). For the third assay, murine splenocyte mitogenesis, the effect of deacylation depended on the source of the LPS. For LPS from *E. coli*, *H. influenzae*, and *P. aeruginosa* and for both smooth and rough *S. typhimurium* LPS, mitogenic activity was only moderately reduced by enzymatic deacylation. In contrast, deacylated *Neisseria* LPS were unexpectedly inactive in this test and could inhibit the ability of untreated homologous and heterologous LPS to stimulate mitogenesis. This finding indicates that the contribution of acyloxyacyl groups to the bioactivity of a given LPS cannot be predicted accurately from the reported behavior of either the corresponding lipid A analog (compound 406) or other deacylated LPS.

Three lines of evidence suggest that the lack of splenocyte mitogenicity of deacylated *Neisseria* LPS is not simply the consequence of toxicity, previously reported to account for the ability of certain lipid A analogs to inhibit splenocyte mitogenesis (38). First, the incorporation of [^{3}H]thymidine by splenocytes incubated with deacylated neisserial LPS was dose dependent, although much lower than expected

TABLE 1. Binding of antibodies to *Neisseria* LPS[a]

Monoclonal antibody	Binding to LPS of			
	N. meningitidis		*N. gonorrhoeae*	
	8693	6155	71H	24-1
3F11	++	+++	+	+
O6B4	++++	++++	+	++
1-1-M	−	−	++++	−
2-1-L8	+	+	+	+
3G9	−	−	++++	+
6B7	+	++++	+	+
1-9C4	++	++++	−	++

[a] The relative expression of each oligosaccharide epitope is designated as follows: ++++, 75 to 100% of maximum-binding control; +++, 50 to 74% of maximum-binding control; ++, 25 to 49% of maximum-binding control; +, 2 to 24% of maximum-binding control; −, <2% of maximum-binding control. Because of the differences in concentrations of each monoclonal antibody and differences in the specific activities and concentrations of the ^{125}I-labeled goat anti-mouse antibodies, comparisons are limited to the binding by a particular monoclonal antibody to different LPS.

(Fig. 2, 4, and 5). Second, the response to concanavalin A, a T-cell mitogen, was not inhibited. Finally, direct measurement of cell viability showed that deacylated LPS was not cytotoxic for B cells.

A better explanation for the loss of mitogenicity might be that enzyme treatment of neisserial LPS resulted in different structural alterations than for other LPS, so that biologic activity was lost to a greater extent. It should be noted, however, that deacylated *Neisseria* LPS retained as much *Limulus* activity as the other deacylated LPS, so the loss of mitogenic activity was not associated with a general loss of bioactivity. An unexpected enzymatic modification of neisserial LPS by AOAH is also unlikely; the enzyme selectively removes secondary acyl chains from all of the LPS studied here (6), and antigenic analysis indicated that enzyme treatment did not remove the oligosaccharide chain from neisserial LPS. It is possible, however, that enzymatic deacylation resulted in a conformational change in the oligosaccharide. While binding of all antibodies was slightly reduced by deacylation (perhaps reflecting reduced adherence of deacylated LPS to the polyvinyl microtiter plates), binding of certain antibodies was affected much more than others (Table 2); this is consistent with previous observations that binding of antibodies 3F11 and 1-1-M is conformation dependent and can be reduced by altering lipid A structure (21, 43).

It is possible that the loss of mitogenicity upon deacylation of neisserial LPS might result from differences in lipid A structure between these LPS and the others used in this work, such as the presence of slightly shorter 3-hydroxy acyl chains at positions 3 and 3' (Fig. 1B). However, *P. aeruginosa* LPS also has two species of hydroxy acyl chains (shorter than those of the enterobacterial LPS) and did not lose mitogenicity when its secondary acyl chains were removed (Fig. 3A).

Although the mitogenic activity of LPS is likely to be primarily a property of the lipid A moiety (since isolated lipid A is mitogenic), there are data indicating that the saccharide chains of LPS may modulate mitogenic activity. It has been reported that enterobacterial LPS with shorter saccharide chains had greater binding to lymphocytes and were more mitogenic than LPS with longer saccharide chains (9, 40). Further, there are reports of mitogenic activity of saccharide chains isolated from *Bordetella pertussis* (8), *H. influenzae* type a (11), and *Bacteroides fragilis* (41). Such activity has not been described for saccharide chains from neisserial LPS, although it is reported that the binding of meningococcal LPS to human monocytes is mediated by the oligosaccharide region (12) and that the isolated chain is able to induce interleukin-1 secretion (2).

The carbohydrate chains of many neisserial LPS contain a structure that is antigenically cross-reactive with the terminal Galβ1→4GlcNAc structures on some human glycosphingolipids; this epitope is recognized by monoclonal antibodies 3F11 and O6B4 (10, 22). Since sphingolipids have been shown to modulate several inflammatory processes (24, 32) and, in particular, to inhibit lymphocyte mitogenesis (16), we considered the possibility that the glycosphingolipidlike structure of some neisserial LPS was involved in the unusual activity of deacylated neisserial LPS. Our data suggest, however, that the loss in mitogenic activity upon deacylation is not a direct consequence of this aspect of the oligosaccharide structure, since it occurred for LPS both with (*N. meningitidis* 8693 and 6155 and *N. gonorrhoeae* 71H and 24-1) and without (*N. meningitidis* 7889 and 126E) the cross-reactive epitope (18, 19, 22) (Table 1).

One explanation for the unexpected removal of mitogenic activity by deacylation of neisserial LPS is that these LPS might possess both mitogenic activity (presumably in the lipid A moiety) and activity inhibitory to mitogenesis (perhaps a property of the oligosaccharide moiety). In intact LPS, the mitogenic effect predominates. Following deacylation, the mitogenic effect of lipid A is reduced, so the net effect would be that the neisserial LPS is essentially inactive and is able to inhibit the mitogenic activity of other LPS. Since mock-treated neisserial LPS is similar to other LPS in mitogenic activity, it is possible that the putative inhibitory

TABLE 2. Enzyme treatment of gonococcal LPS does not destroy all antibody-binding sites

Monoclonal antibody	*N. gonorrhoeae* 71H			*N. gonorrhoeae* 24-1		
	LPS	dLPS[a]	(% Reduction)	LPS	dLPS	(% Reduction)
3F11	7,465[b]	5,647	25	8,875	3,037	66
O6B4	7,855	4,825	39	39,457	19,927	50
1-1-M	16,033	7,777	52	961	1,171	0
2-1-L8	1,585	1,519	4	1,543	1,117	28
3G9	107,917	89,071	18	4,111	1,801	57
6B7	5,023	3,865	24	3,901	3,649	7
1-9C4	583	769	0	8,791	6,475	27

[a] dLPS, enzyme-treated LPS.
[b] Counts per minute of radiolabeled goat anti-mouse immunoglobulin G or immunoglobulin M antibody (specific activity, approximately 1,500 cpm/ng) bound to LPS-coated wells.

activity is not present in intact LPS; it might be latent, and come into effect after deacylation, perhaps as a result of a conformational change. The mitogenic effect of the lipid A moiety is presumably directed toward B cells; however, the inhibitory activity might be directed toward a different cell population. It is also possible that the B-cell mitogenic activity of neisserial LPS might differ from that of enterobacterial LPS in having a greater dependence on a signal from another target cell population, such as mononuclear phagocytes, that might not respond to deacylated LPS. Our data do not show whether the inhibitory effect of deacylated *Neisseria* LPS extends to other B-cell mitogens.

It is evident from these studies that the contribution of acyloxyacyl groups to the bioactivity of a given LPS structure cannot be predicted with confidence from the behavior of either lipid A analogs or other deacylated LPS. The observation that deacylated meningococcal LPS inhibits the splenocyte mitogenic activity of intact LPS is consistent with numerous observations that LPS or lipid A analogs that are not active in a particular LPS response often are inhibitory (20, 28, 29, 35) and with previous observations that enzymatically deacylated *S. typhimurium* Rc LPS inhibits some LPS activities (4, 27, 30). This appears to be the first example of a lipid A or LPS partial structure that can inhibit LPS-induced murine splenocyte mitogenesis, however. Exploration of the structural basis and biological significance of the unusual behavior of deacylated *Neisseria* LPS should be an interesting avenue for future investigation.

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