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Endotoxic Induction of Prostaglandin Release from Macrophages by Nontoxic Lipid A Analogs Synthesized Chemically

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The ability of synthetic lipid A analogs to induce prostaglandin synthesis in macrophages was compared with that of native lipopolysaccharide. The synthetic preparations comprised monomeric or dimeric derivatives of D-glucosamine with different patterns of substitution by phosphate and tetradecanoic, (*R*)-3-hydroxytetradecanoic, and (*R*)-3-tetradecanoyloxytetradecanoic acid. All of these preparations are structurally distinct from native lipid A (principally regarding the position of fatty acid substitution) and hence have been previously shown to be endotoxically inactive in many biological tests. It was found that many of these synthetic samples exhibit strong activity in inducing prostaglandin E_2 and prostaglandin $F_2\alpha$, with some of them having activity comparable to that of lipopolysaccharide. Experiments with macrophages of C3H/HeJ mice enabled us to differentiate between endotoxin-specific (in the case of dimeric preparations) and endotoxin-nonspecific (for monomeric preparations) mechanisms for the induction of prostaglandins. These results indicate that there is a difference in the mechanism of induction of prostaglandin synthesis between monomeric lipid A's and dimeric or native lipid A structures.

Lipid A, which is the lipid part of lipopolysaccharide (LPS), has been thought to be the active center of endotoxin (23). The chemical structure of lipid A from *Escherichia coli* was recently determined, and the lipid was subsequently synthesized chemically (10, 11, 22). This compound exhibited the same activities as those of natural lipid A in many assay systems so far tested (6, 9, 20). These results showed that the proposed chemical structure of lipid A is correct and that lipid A alone is the active center of almost all the biological activities of endotoxin. The chemically synthesized compounds not only contributed to the confirmation of the above findings but also enabled us to study precisely the relationship between chemical structure and biological activities (4–6, 9, 16–20). It is concluded that all three constituents of the lipid A structure—that is, a diglucosamine backbone, phosphate, and fatty acids—are prerequisite for the full expression of endotoxic activities. Among these constituents, the position of fatty acid substitution was especially critical for endotoxic activities (4, 16, 19). Thus the compounds whose fatty acids were substituted at positions 2, 3, and 4 of a reducing glucosamine residue and positions 2 and 6 of a nonreducing glucosamine residue and which were first synthesized after the old model of lipid A structure were almost inactive compared with natural lipid A (29, 30).

We show here, however, that some of these preparations surprisingly stimulate macrophages to induce prostaglandin synthesis in culture to an extent comparable to that evoked by native LPS.

Prostaglandins are presumed to act as mediators of certain LPS actions such as induction of fever (1, 27), abortion (28), and shock (2, 3, 8), and the macrophage is probably one of the most important cellular targets of endotoxin (21, 25). Therefore, the ability of a compound to induce prostaglandin synthesis in macrophages in vitro seemed to be a good indicator for the structural requirements of cell activation. Moreover, the use of macrophages of tolerant mice makes it possible to distinguish between an endotoxic and a more general signal (31).

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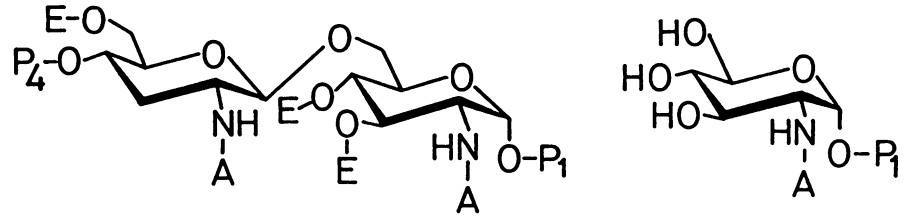
MATERIALS AND METHODS

Stimulants of prostaglandin release. Lipopolysaccharide (LPS) from *Salmonella minnesota* R595 (7) and the synthetic lipid A analogs (12–14) have been described previously (30). The chemical structures of the synthetic preparations used in this study are shown in Fig. 1. Some of the synthetic (phosphate-containing) preparations were solubilized or finely suspended in water after conversion into triethylammonium salt by shaking in 0.1 N HCl at 0°C for 2 to 5 min, followed by centrifugation, washing, suspension in water, ultrasonication, and neutralization with triethylamine. Zymosan was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Animals. Female, 6- to 8-week-old NMRI mice (Ivanovas), 10-week-old C3H/HeJ mice (Max-Planck-Institut für Immunbiologie, Freiburg, Federal Republic of Germany), and Gelb-Silber rabbits (Borstel breed) were used.

Preparation of cells. Mouse peritoneal cells were obtained by washing the peritoneal cavity with 5 ml of Iscove medium containing L-glutamine and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (GIBCO Laboratories, Grand Island, N.Y.) (26). The cell number was adjusted to 5×10^5 cells per well. Rabbit alveolar macrophages were harvested by lavaging the lungs with 20 ml of phosphate-buffered saline three to four times after the animals had been sacrificed by intravenous injection of 10 ml of air. About 1×10^8 to 2×10^8 cells were obtained from each rabbit; 10^5 cells per ml per well were used for the tests. These macrophages were allowed to adhere for 3 h at 37°C. After the cells were washed three times with phosphate-buffered saline (37°C), 1 ml of Iscove medium was added to each well and served for the tests.



Sample number	Fatty acid E	Fatty acid A	Phosphate P_1	Phosphate P_4
301	C_{14}	C_{14}		
304	C_{14}	C_{14}		P_4
303	C_{14}	C_{14}	P_1	
305	C_{14}	C_{14}	P_1	P_4
307	H	C_{14}-OH		
302	C_{14}	C_{14}-OH		
321	C_{14}	C_{14}-OH		P_4
316	C_{14}	C_{14}-OH	P_1	
317	C_{14}	C_{14}-OH	P_1	P_4
314	C_{14}OH	C_{14}-O-C_{14}		

Sample number	Fatty acid A	Phosphate P_1
309	C_{14}	
308	C_{14}-OH	
318	C_{14}-OH	P_1
313	C_{14}-O-C_{14}	

FIG. 1. Chemical structures of the synthetic lipid A analogs of disaccharide and monosaccharide derivatives. Abbreviations: E and A, Ester- and amide-linked fatty acids, respectively; P_1 and P_4, phosphate groups at positions 1(α) and 4', respectively; C_{14}, tetradecanoyl; C_{14}-OH, (*R*)-3-hydroxytetradecanoyl; C_{14}-O-C_{14}, (*R*)-3-tetradecanoyloxytetradecanoyl.

Induction of prostaglandin release and its determination. Macrophages were incubated with different stimulants for 24 h (37°C). The cells were then centrifuged, and the prostaglandins in the supernatant were determined by radioimmunoassay (15). All experiments were done in duplicate. Anti-prostaglandin $F_2\alpha$ ($PGF_2\alpha$) antisera were prepared by the method of Pesker et al. (24). Antisera against prostaglandin E_2 (PGE_2) were purchased from Sigma. [^{3}H]PGE_2 and [^{3}H]$PGF_2\alpha$ were purchased from Dupont, NEN Research Products (Boston, Mass.) (specific activities of PGE_2 and $PGF_2\alpha$ were 165.0 and 150.0 Ci/mmol, respectively).

RESULTS

Induction of prostaglandin release from rabbit alveolar macrophages by synthetic lipid A analogs. Rabbit alveolar macrophages were cultured, and prostaglandin was determined as described in Materials and Methods. With some of the lipid A analogs (compounds 303, 305, 307, and 316), the relationship between dose and the amount of $PGF_2\alpha$ released was studied (Fig. 2). All the tested substances provoked prostaglandin release, but to different extents. Thus, with 30 μg of preparation 303, 9, ng of $PGF_2\alpha$ per ml was detected, whereas with preparation 316, only 1.8 ng/ml was found.

Disaccharide preparations 303 and 305, which are substituted with myristic acid in the ester and amide linkage and a phosphate group at C1 (303) and at C1 and C4 (305), induced 10.8 and 10.4 ng of $PGF_2\alpha$, respectively; these amounts were comparable to those evoked by native LPS (12.1 ng). Significant but smaller amounts of prostaglandins were also induced by compounds 304 (2.8 ng), 307 (5.0 ng), 314 (2.9 ng), 316 (3.3 ng), and 317 (3.1 ng).

The monosaccharide derivatives 308 (3.8 ng) and 313 (2.6 ng) also exhibited moderate activity (Table 1).

Induction of prostaglandin release from peritoneal macrophages of LPS-responder mice by synthetic lipid A analogs. The effect of synthetic lipid A analogs (compounds 303, 304, 313, and 316) on mouse peritoneal macrophages is shown in Fig. 3. Like rabbit alveolar macrophages, mouse peritoneal cells responded to all compounds tested in a dose-dependent manner (Fig. 3). Compound 303 exhibited activity similar to that in alveolar macrophages. Compound 316, however, which exhibited low activity in alveolar macrophages (Fig. 2), was very active in the induction of $PGF_2\alpha$ in mouse

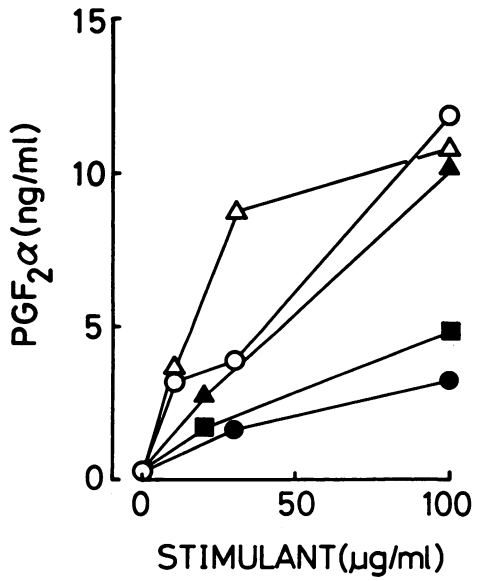


FIG. 2. Dose responses of LPS and synthetic lipid A analogs for induction of $PGF_2\alpha$ release by rabbit alveolar macrophages; 10^5 cells were used for the tests. The stimulants shown are LPS from *S. minnesota* R595 (○) and synthetic preparations 303 (△), 305 (▲), 307 (■), and 316 (●).

TABLE 1. Induction of prostaglandin release from mouse peritoneal and rabbit alveolar macrophages by synthetic lipid A analogs

Compound	Sample no.	Solubility[a]	Dose (μg/ml)	Prostaglandin release (pg/ml) from macrophages				
				Rabbit alveolar		Mouse peritoneal		
						NMRI		C3H/HeJ (E_2)
				$F_2\alpha$	E_2	$F_2\alpha$	E_2	
Disaccharide derivatives	301	−	100	340	50	1,340	105	ND[b]
	304	+	100	2,790	1,230	5,400	6,200	336
	303	+	100	10,800	6,820	11,700	11,000	2,550
	305	+	100	10,400	6,760	ND	3,900	217
	307	+	100	4,970	1,560	4,740	8,000	502
	302	−	100	250	69	2,540	ND	ND
	321	+	30	830	744	3,200	ND	ND
			100	ND	ND	ND	4,600	1,230
	316	+	100	3,260	ND	10,300	4,600	454
	317	++	30	3,080	1,230	2,200	ND	ND
			100	ND	ND	ND	2,300	273
	314	−	100	2,920	1,240	1,610	30	ND
Monosaccharide derivatives	309	−	100	356	45	1,950	50	ND
	308	−	100	3,750	1,170	5,320	3,800	3,300
	318	++	100	410	ND	2,030	ND	ND
	313	−	50	2,560	1,170	ND	ND	ND
			100	ND	ND	12,000	2,000	5,070
LPS (R595)		++	100	12,100	ND	12,200	6,600	201
Zymosan	−	500	ND	ND	13,300	6,900	6,260	
Control		0	225	34	1,350	208	203	

[a] ++, Very soluble; +, soluble; −, insoluble.
[b] ND, Not determined.

peritoneal macrophages. Table 1 summarizes the prostaglandin induction capacities of several synthetic substances. The capacity for induction of PGE_2 and $PGF_2\alpha$ by compounds 303 (11.7 and 11.0 ng of $PGF_2\alpha$ and PGE_2, respectively), 316 (10.3 and 4.6 ng of $PGF_2\alpha$ and PGE_2, respectively), and 313 (12.0 and 2.0 ng of $PGF_2\alpha$ and PGE_2, respectively), at doses of 100 μg/ml, was comparable to that by LPS (12.2 and 6.6 ng of $PGF_2\alpha$ and PGE_2, respectively). Less but significant activity was also exhibited by compounds 304, 305, 307, 317, 321, and 308, whereas no prostaglandin induction could be observed with preparations 301, 302, 314, 309, and 318.

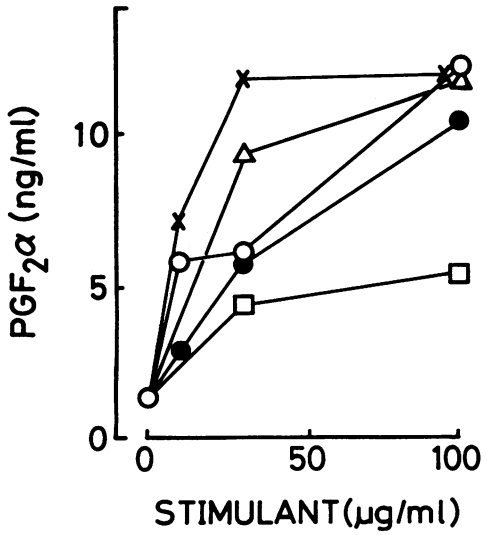


FIG. 3. Dose responses of LPS and synthetic lipid A analogs for induction of $PGF_2\alpha$ release by mouse peritoneal macrophages; the 5×10^5 cells were used for the tests. The stimulants shown are LPS from *S. minnesota* R595 (○) and synthetic preparations 303 (△), 304 (□), 313 (X), and 316 (●).

Induction of prostaglandin release from peritoneal macrophages of LPS-nonresponder mice by synthetic lipid A analogs. Macrophages of the LPS-nonresponder C3H/HeJ mice differ from those of normal, LPS-responder mice in that they cannot be activated by LPS to produce increased levels of prostaglandins (31). However, when activated with other stimuli, e.g., zymosan, they respond with prostaglandin synthesis (Table 1). This pattern of response makes it possible to distinguish between the activation of these cells by the endotoxin structure and nonspecific activation. LPS at a concentration of 100 μg/ml had no effect on these cells. Some of the synthetic structures had a stimulating, hence nonspecific, effect on C3H/HeJ macrophages (compound 308, 3.3 ng; compound 313, 5.1 ng). On the other hand, preparations 304, 305, 307, 316, and 317 (each with a strong ability to activate normal cells) did not have this effect on C3H/HeJ macrophages.

DISCUSSION

After the chemical structure of lipid A of *E. coli* was established and the synthetic lipid A of the *E. coli* type was proved to be biologically identical to the natural compound in many tests, one of the questions remaining was whether the variety of endotoxic activities could be separated structurally. By using several synthetic lipid A analogs, the relationship between chemical structure and biological activities was precisely studied (4–6, 9, 16–20, 29, 30). It was found that for the full expression of all endotoxic activities, each of the three constituents of the lipid A structure—the diglucosamine backbone, phosphate, and fatty acids—plays an important role. However, some of the activities were also

exhibited by the incomplete form of lipid A. It could be generally said that a more complete structure of lipid A was required for toxic activities (such as pyrogenicity or the Shwartzman reaction) than for beneficial ones (such as mitogenicity, adjuvanticity, and tumor necrosis factor or interferon induction), which were expressed by even a very simple form of lipid A.

These facts suggest that substances which possess beneficial activity without toxicity can be obtained. In this regard, it seemed interesting to test some biological activities of the compounds which resemble the native compound structurally but are biologically nontoxic. The synthetic lipid A analogs used in these experiments were previously found to be almost inactive compared with native LPS or lipid A because the position of fatty acid substitution was incorrect (29, 30). Surprisingly, however, some of these compounds, when used to induce prostaglandin synthesis, were found to exhibit activities that nearly equaled that of native LPS.

In order to control the specificity of the endotoxic natures of the synthetic compounds, the compounds were incubated with macrophages of the LPS-nonresponder mouse strain C3H/HeJ. Because all of the soluble compounds having a dimeric glucosamine backbone exhibited only low activity compared with zymosan, it is concluded that they have structural designs resembling that of native lipid A. However, none of the monomeric derivatives was endotoxically active, as they stimulated macrophages of responder mice as well as of nonresponder mice.

The structural requirements of lipid A for the induction of prostaglandins are not very strict. The phosphate molecule does not seem to play a direct role because preparation 307, which has no phosphate, showed strong activity. However, the phosphate molecule contributed to the solubility of the samples. All of the insoluble compounds, as judged by visible solubility behavior, were inactive or had very low activity (compounds 301, 302, and 314), whereas when phosphate was introduced into the molecule to promote solubility, the activity increased (compound 301 [no phosphate], inactive; compound 304 [P4], moderately active; and compounds 303 [P1] and 305 [P1 and P4], highly active). The activity of the compounds was demonstrated regardless of the number or kind of fatty acids substituted. Two amide-linked fatty acids in the molecule are enough to induce activity (307); 3-hydroxymyristic acid is not necessary, as was shown by compounds 303, 304, and 305, whose fatty acids consist only of myristic acids. The position of the substituted fatty acid, which was previously found to be absolutely decisive for the other endotoxic activities, did not have any influence on this activity. The results obtained here clearly indicate that one of the endotoxic activities could be separated structurally from the others.

LITERATURE CITED

1. **Cranston, W. I.** 1979. Central mechanism of fever. Fed. Proc. **38**:49–51.
2. **Fletcher, J. R., and P. W. Ramwell.** 1978. *E. coli* endotoxin shock in the baboon: treatment with lidocaine or indomethacin, p. 183–192. *In* C. Galli, G. Galli, and G. Porcellati (ed.), Advances in prostaglandin and thromboxane research, vol. 3. Raven Press, New York.
3. **Flynn, J. T.** 1978. Endotoxin shock in the rabbit: the effects of prostaglandin and arachidonic acid administration. J. Pharmacol. Exp. Ther. **206**:555–566.
4. **Galanos, C., V. Lehmann, O. Lüderitz, E. T. Rietschel, O. Westphal, H. Brade, L. Brade, M. A. Freudenberg, T. Hansen-Hagge, T. Lüderitz, G. McKenzie, U. Shade, W. Stritmatter, K. Tanamoto, U. Zähringer, M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba.** 1984. Endotoxic properties of chemically synthesized lipid A part structures. Comparison of synthetic lipid A precursor and synthetic analogues with biosynthetic precursor and free lipid A. Eur. J. Biochem. **140**:221–227.
5. **Galanos, C., O. Lüderitz, M. Freudenberg, L. Brade, U. Shade, E. T. Rietschel, S. Kusumoto, and T. Shiba.** 1986. Biological activity of synthetic heptaacyl lipid A representing a component of *Salmonella minnesota* R 595 lipid A. Eur. J. Biochem. **160**:55–59.
6. **Galanos, C., O. Lüderitz, E. T. Rietschel, O. Westphal, H. Brade, L. Brade, M. Freudenberg, U. Shade, M. Imoto, H. Yoshimura, S. Kusumoto, and T. Shiba.** 1985. Synthetic and natural *Escherichia coli* free lipid A express identical endotoxic activities. Eur. J. Biochem. **148**:1–5.
7. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9**:245–249.
8. **Hilton, J. G., and C. H. Wells.** 1976. Effects of indomethacin and nicotinic acid on *E. coli* endotoxic shock in anesthetized dogs. J. Trauma **16**:968–973.
9. **Homma, J. Y., M. Matsuura, S. Kanegasaki, Y. Kawakubo, Y. Kojima, N. Shibukawa, Y. Kumazawa, A. Yamamoto, K. Tanamoto, T. Yasuda, M. Imoto, H. Yoshimura, S. Kusumoto, and T. Shiba.** 1985. Structural requirements of lipid A responsible for the functions: a study with chemically synthesized lipid A and its analogues. J. Biochem. **98**:395–406.
10. **Imoto, M., S. Kusumoto, T. Shiba, H. Naoki, T. Iwashita, E. T. Rietschel, H. Wollenweber, C. Galanos, and O. Lüderitz.** 1983. Chemical structure of *E. coli* lipid A: linkage site of acyl groups in the disaccharide backbone. Tetrahedron Lett. **24**:4017–4020.
11. **Imoto, M., H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba.** 1985. Total synthesis of *Escherichia coli* lipid A. Tetrahedron Lett. **26**:1545–1548.
12. **Inage, M., H. Chaki, S. Kusumoto, and T. Shiba.** 1980. Synthesis of lipopolysaccharide corresponding to fundamental structure of *Salmonella*-type lipid A. Tetrahedron Lett. **21**:3889–3892.
13. **Inage, M., H. Chaki, S. Kusumoto, and T. Shiba.** 1981. Chemical synthesis of phosphorylated fundamental structure of lipid A. Tetrahedron Lett. **22**:2281–2282.
14. **Inage, M., H. Chaki, S. Kusumoto, T. Shiba, A. Tai, M. Nakahata, T. Harada, and Y. Izumi.** 1980. Chemical synthesis of bisdephospho lipid A of *Salmonella* endotoxin. Chem. Lett. **1980**:1373–1376.
15. **Jobke, A., B. A. Pesker, and B. M. Pesker.** 1973. On the specificity of antisera against prostaglandin A and E. FEBS Lett. **37**:192–196.
16. **Kanegasaki, S., Y. Kojima, M. Matsuura, J. Y. Homma, A. Yamamoto, Y. Kumazawa, K. Tanamoto, T. Yasuda, T. Tsumita, M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba.** 1984. Biological activities of analogues of lipid A based on the revised structural model. Eur. J. Biochem. **143**:237–242.
17. **Kanegasaki, S., K. Tanamoto, T. Yasuda, J. Y. Homma, M. Matsuura, M. Nakatsuka, Y. Kumazawa, A. Yamamoto, T. Shiba, S. Kusumoto, M. Imoto, H. Yoshimura, and T. Shimamoto.** 1986. Structure-activity relationship of lipid A: comparison of biological activities of natural and synthetic lipid A's with different fatty acid compositions. J. Biochem. **99**:1203–1210.
18. **Kotani, S., H. Takada, I. Takahashi, M. Tsujimoto, T. Ogata, T. Ikeda, K. Harada, H. Okumura, T. Tamura, S. Tanaka, T. Shiba, S. Kusumoto, M. Imoto, H. Yoshimura, and N. Kasai.** 1986. Low endotoxic activities of synthetic *Salmonella*-type lipid A with an additional acyloxyacyl group on the 2-amino group of β(1-6)glucosamine disaccharide 1,4′-bisphosphate. Infect. Immun. **52**:872–884.
19. **Kotani, S., H. Tanaka, M. Tsujimoto, T. Ogata, Y. Mori, A. Kawasaki, A. Tanaka, S. Nagao, S. Tanaka, T. Shiba, S. Kusumoto, M. Imoto, H. Yoshimura, M. Yamamoto, and T. Shimamoto.** 1984. Immunobiologically active lipid A analogs synthesized according to a revised structural model of natural lipid A. Infect. Immun. **45**:293–296.

20. **Kotani, S., H. Tanaka, M. Tsujimoto, T. Ogata, I. Takahashi, T. Ikeda, K. Otsuka, H. Shimauchi, N. Kasai, J. Mashimo, S. Nagao, A. Tanaka, S. Tanaka, K. Harada, K. Nagaki, H. Kitamura, T. Shiba, S. Kusumoto, M. Imoto, and H. Yoshimura.** 1985. Synthetic lipid A with endotoxic and related biological activities comparable to those of a natural lipid A from an *Escherichia coli* Re-mutant. Infect. Immun. **49**:225–237.
21. **Kurland, J. I., and R. Bockman.** 1978. Prostaglandin E production by human blood monocytes and mouse peritoneal macrophages. J. Exp. Med. **147**:952–957.
22. **Kusumoto, S., H. Yoshimura, M. Imoto, T. Shimamoto, and T. Shiba.** 1985. Chemical synthesis of 1-dephospho derivatives of *Escherichia coli* lipid A. Tetrahedron Lett. **26**:909–912.
23. **Lüderitz, O., M. Freudenberg, C. Galanos, V. Lehmann, E. T. Rietschel, and D. H. Show.** 1982. Lipopolysaccharides of gram-negative bacteria. Curr. Top. Membr. Transp. **17**:79–151.
24. **Pesker, B. A., H. Anhut, E. E. Kroner, and B. M. Pesker.** 1979. Development, specificity and some applications of radioimmunoassays for prostaglandins and related compounds, p. 275–286. *In* J. P. Tillement (ed.), Advances in pharmacology and therapeutics, vol. 7. Biochemical clinical pharmacology. Pergamon Press, Inc., Elmsford, New York.
25. **Rietschel, E. T., U. Shade, O. Lüderitz, H. Fisher, and B. A. Pesker.** 1980. Prostaglandins in endotoxicosis, p. 66–72. *In* D. Schlessinger (ed.), Microbiology—1980. American Society for Microbiology, Washington, D.C.
26. **Shade, U., and E. T. Rietschel.** 1980. Differences in lipopolysaccharide-induced prostaglandin release and phagocytic activity of peritoneal macrophages from LPS-hypersensitive and tolerant mice, p. 271–277. *In* D. Eaker and T. Wadstrom (ed.), Natural toxins. Pergamon Press, Inc., Elmsford, N.Y.
27. **Siegert, R., W. K. Philipp-Dormston, K. Radsak, and H. Menzel.** 1976. Mechanism of fever induction in rabbits. Infect. Immun. **14**:1130–1137.
28. **Skarnes, R. C., and M. J. K. Harper.** 1972. Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. Prostaglandins **1**:191–203.
29. **Tanamoto, K., C. Galanos, O. Lüderitz, S. Kusumoto, and T. Shiba.** 1984. Mitogenic activities of synthetic lipid A analogs and suppression of mitogenicity of lipid A. Infect. Immun. **44**: 427–433.
30. **Tanamoto, K., U. Zähringer, G. McKenzie, C. Galanos, E. T. Rietschel, O. Lüderitz, S. Kusumoto, and T. Shiba.** 1984. Biological activities of synthetic lipid A analogs: pyrogenicity, lethal toxicity, anticomplement activity, and induction of *Limulus* amoebocyte lysate. Infect. Immun. **44**:421–426.
31. **Wahl, L. M., D. L. Rosenstreich, L. M. Glode, A. L. Sandberg, and S. E. Mergenhagen.** 1979. Defective prostaglandin synthesis by C3H/HeJ mouse macrophages stimulated with endotoxin preparations. Infect. Immun. **23**:8–13.