Mitogenic Activities of Synthetic Lipid A Analogs and Suppression of Mitogenicity of Lipid A

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The effect of synthetic lipid A analogs on murine spleen cells was studied. The preparations represented d-glucosamine and d-glucosaminyl-β1,6-d-glucosamine disaccharide derivatives substituted in different combinations by ester- and amide-bound fatty acids and by phosphate groups. Significant mitogenic activity was demonstrated with a number of synthetic disaccharide preparations; however, their potency was lower than that of lipid A. The synthetic preparations were not mitogenic for spleen cells from C3H/HeJ mice. Furthermore, the mitogenicity of the synthetic preparations was abolished after binding with lipid A and B. The results indicate that for expression of mitogenicity, a phosphate group at position 1 of the reducing glucosamine and amide-bound acyloxyacyl residues are important factors. Some of the synthetic preparations containing the diglucosamine backbone and expressing relatively low mitogenicity suppressed B-cell mitogenicity of lipid A. Although these preparations were lytic for erythrocytes, they did not affect the viability of the splenic lymphocytes. Suppression was seen when the synthetic preparations were added simultaneously with or after the lipid A mitogen, but optimal suppression was expressed when the synthetic preparations were added to the system 3 h before lipid A. Washing of the cells before the addition of lipid A did not affect the results. The suppression was not due to the induction of suppressor cells by the synthetic preparations. The disaccharide preparations did not inhibit T-cell mitogenicity of concanavalin A. In contrast to the disaccharide preparations, the monosaccharide preparations suppressed mitogenicity of both lipid A and concanavalin A, probably because of their direct toxicity for lymphocytes.

In a previous study (23) synthetic lipid A analogs were tested for several endotoxic activities, and some were found to express pyrogenicity, lethal toxicity, and anticomplement activity. None of them was active in the Limulus gelatin test. In the present work, the action of the synthetic preparations on murine spleen cells was studied. Lipopolysaccharides exert a wide range of effects on the immune system. They are known to act as potent B-cell mitogens (1–3, 8, 20, 22), and it is generally accepted that lipid A is responsible for this activity (2, 3, 19, 22). We tested the B-cell mitogenic activity of the synthetic lipid A analogs to determine possible structure-activity relationships. During these investigations it was found that some synthetic preparations suppressed the mitogenic activity of natural lipid A and that some preparations would lyse erythrocytes and/or be toxic for lymphocytes. The studies described here were performed with either the original synthetic preparations or, in the case of insolubility, with the succinylated derivatives.

MATERIALS AND METHODS

Materials. Salmonella minnesota S and Re lipopolysaccharides, free lipid A from S. minnesota Re (6, 7), the synthetic lipid A analogs (10–12), and the succinylated derivatives are described in an accompanying paper (23). The structures and designations of the synthetic preparations are shown in Fig. 1 of that paper (23).

Preparation of cells. Spleen cells from female BALB/c or C3H/HeJ mice (6 to 10 weeks old) were pressed through a wire grid and suspended in Iscove modified Dulbecco medium containing L-glutamine and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (GIBCO Laboratories, Grand Island, N.Y.) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). The cell suspension was washed three times with the medium.

Cell culture and assay of mitogenicity. Cells were cultured in Iscove medium as described above on microplates (Falcon Plastics, Oxnard, Calif.) at 37°C with 10% CO2. Each well contained 8 × 106 cells in 200 μl and the mitogen. After 2 days of incubation, [3H]thymidine (0.2 μCi per culture; Amersham International Ltd., Amersham, England) was added. After 24 h the cultures were harvested (multiple sample harvester; Skapron A. S., Lierbyen, Norway), collected on glass fiber filters (Titertek, Uxbridge, England), and washed with distilled water. The filters were dried and transferred to scintillation tubes. Xylo1-based scintillation fluid (0.4 ml) was added, and radioactivity was measured in a scintillation spectrometer (TriCarb; Packard, Instrument Co., Inc., Rockville, Md.). All experiments were done in triplicate. Because of daily variations, reference lipid A was included in each test. Mitogenic activity of the preparations is expressed as either a percentage of maximum counts per minute incorporation based on incorporated radioactivity with lipid A (100%) or as parallel test or as an index based on the background incorporation without mitogen taken as 1.

Inhibition of lipid A mitogenicity by synthetic lipid A analogs. The synthetic preparations were added to the assay system before, together with, or after the mitogen, which was used in a dose leading to optimal stimulation (10 μg/ml for lipid A and 0.6 μg/ml for concanavalin A [ConA]). The suppression of [3H]thymidine incorporation was tested in comparison with a control containing mitogen only.

Hemolytic activity of synthetic preparations and toxicity for spleen lymphocytes. Sheep erythrocytes (4 × 108 cells per ml) and test samples were incubated for different periods of time in Falcon plastic tubes at 37°C in the presence of 10% CO2. The remaining cells were counted directly.

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Polymyxin B interaction with synthetic lipid A analog (14).

**RESULTS**

Mitogenic effects of lipopolysaccharides and lipid A. Figure 1 shows the mitogenic activity of *S. minnesota* S- and Re-form lipopolysaccharides and of free lipid A. Stimulation of spleen cells of mice to increased DNA synthesis was seen with all preparations, but the degree of maximum stimulation was different. Also, the dose responses of the three mitogens were different. S-form lipopolysaccharide expressed the highest stimulating effect at the highest concentration tested (100 μg/ml); the Re mutant lipopolysaccharide had maximum activity at 10 to 30 μg/ml, and at higher concentrations the activity decreased gradually. For free lipid A, optimum stimulation occurred at 10 μg/ml, and a drastic decrease of activity was seen at higher concentrations.

For succinylated lipid A (Fig. 1) the dose of optimum stimulation (10 μg/ml) was the same as for lipid A, but the overall thymidine incorporation was low compared with natural lipid A (35%); at higher doses, as with lipid A, decreased thymidine incorporation was observed.

Mitogenicity of synthetic lipid A analogs. The results of mitogenicity tests with the synthetic lipid A analogs are shown in Table 1. The data are derived from several experiments, each of which was done in triplicate. The samples exhibited mitogenic activity of different degrees, but they all were less active than natural lipid A. For some preparations optimal stimulation was not obtained even with the highest concentration of sample employed (100 μg/ml). Some preparations showed a decrease of activity at concentrations.

![Graph](image)

**TABLE 1. Mitogenicity of synthetic lipid A analogs**

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Original (orig) or succinylated (succ)</th>
<th>Solubility</th>
<th>OMD (μg/ml)</th>
<th>Mitogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>301</td>
<td>succ</td>
<td>+</td>
<td>25</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td>304</td>
<td>orig</td>
<td>-</td>
<td>100</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>303</td>
<td>orig</td>
<td>+</td>
<td>100</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>305</td>
<td>orig</td>
<td>+</td>
<td>30</td>
<td>38.2 ± 12.2</td>
</tr>
<tr>
<td>302</td>
<td>succ</td>
<td>+</td>
<td>100</td>
<td>6.9 ± 1.9</td>
</tr>
<tr>
<td>312</td>
<td>orig</td>
<td>+</td>
<td>100</td>
<td>6.7 ± 1.9</td>
</tr>
<tr>
<td>316</td>
<td>succ</td>
<td>+</td>
<td>100</td>
<td>21.2 ± 3.3</td>
</tr>
<tr>
<td>317</td>
<td>orig</td>
<td>+</td>
<td>100</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>311</td>
<td>succ</td>
<td>+</td>
<td>100</td>
<td>10.3 ± 1.2</td>
</tr>
<tr>
<td>310</td>
<td>orig</td>
<td>+</td>
<td>100</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td>314</td>
<td>succ</td>
<td>+</td>
<td>100</td>
<td>7.8 ± 2.4</td>
</tr>
<tr>
<td>315</td>
<td>orig</td>
<td>+</td>
<td>100</td>
<td>6.6 ± 1.5</td>
</tr>
<tr>
<td>318</td>
<td>succ</td>
<td>+</td>
<td>100</td>
<td>5.1 ± 1.1</td>
</tr>
<tr>
<td>319</td>
<td>orig</td>
<td>+</td>
<td>100</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>313</td>
<td>succ</td>
<td>+</td>
<td>100</td>
<td>19.1 ± 7.2</td>
</tr>
</tbody>
</table>

*Mitogenic index = maximum cpm of test sample - cpm of background / cpm of background.*

*Percent mitogenicity based on lipid A = maximum cpm of test sample - cpm of background / maximum cpm of lipid A - cpm of background.*

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**Notes:**

- **Structures of the synthetic preparations are given in Fig. 1 of reference 23.**
- **OMD, Optimal mitogenic dose.**
- **+ + solution; +, fine suspension; -, particles.**
- **Percent mitogenicity based on lipid A = maximum cpm of test sample - cpm of background / maximum cpm of lipid A - cpm of background.**
- **Mitogen index = maximum cpm of test sample / cpm of background.**
above optimum. Dose-response curves with preparations 303 and 316 are shown in Fig. 2 together with that of lipid A. The mitogenic index was about 4 for both samples, and they exhibited about 38 and 21% of the activity expressed by natural lipid A, respectively. As with lipid A, a drastic decrease of mitogenicity to 0 was observed after succinylation of 316. Succinylated preparations 314 and 315, containing amide-bound acyloxyacyl residues, showed relatively strong mitogenicity. None of the monosaccharide preparations exhibited mitogenic activity.

Samples 303 and 316 were tested with spleen cells from C3H/HeJ mice. No mitogenic stimulation was seen with either sample (Table 2).

The mitogenicity of lipopolysaccharides is known to be abolished by complexation with polymyxin B (14). When synthetic preparation 316 and lipid A as a reference were complexed with polymyxin B, mitogen responses were completely inhibited (data not shown).

**Suppression of mitogenic activity of lipid A and ConA by synthetic preparations.** It was found that some of the synthetic preparations suppressed the mitogenicity of natural lipid A when they were added to the culture of spleen cells together with the mitogen. An optimal concentration (10 μg/ml) of lipid A was always used for stimulation. As shown in Fig. 3, preparation 316 did not interfere with the mitogenicity of lipid A. The addition of preparation 303 to the system resulted in an increased incorporation (by 40%) of radioactivity. The other synthetic lipid A analogs, including the monosaccharide derivatives, showed suppressive activity, but to different degrees. Suppression was also observed with high amounts of natural lipid A (compare Fig. 1) and its succinylated form (data not shown).

As shown in Fig. 4, the T-cell mitogenic activity of ConA was not suppressed by the synthetic disaccharide preparations up to a concentration of 100 μg/ml. In contrast, the monosaccharide preparations expressed strong suppression of the mitogenicity of ConA.

The effect of varying the time of addition of the synthetic preparations relative to the addition of lipid A to the cultures was studied. Lipid A (10 μg/ml) was added to the culture at time zero, and the synthetic preparations (100 μg/ml) were added at −3, 0, 3, 24, and 48 h. Four samples, disaccharide preparations 321 and succinylated 302 and monosaccharide preparations 318 and succinylated 313, were tested. The results are shown in Fig. 5. Addition to the spleen cells of preparations 302 and 321 h before the addition of lipid A was most effective in suppressing mitogenicity. The results were not changed when the cells were washed before the addition of lipid A. The effects were smaller when the preparations were added after lipid A, and at 48 h no suppression was observed. In contrast, monosaccharide preparations 318 and succinylated 313 showed at all times of addition a strong suppression of lipid A mitogenicity, and similarly, the mitogenicity of ConA was abolished. In the latter system, disaccharide preparation 321 had no effect at any time of addition (data not shown). To examine whether the synthetic lipid A analogs would induce the formation of suppressor cells, spleen cells were preincubated with the synthetic preparations and the effect of these cells on normal spleen cells was tested. Succinylated preparations 302 (100 μg/ml) and 313 (10 μg/ml) were incubated for 3 h with spleen cells, and the cells were washed with medium. Treated and normal cells were mixed in different ratios (1:0, 1:1, 1:9, and 0:1) and adjusted to a final cell number of 4 × 10⁶ cells per ml. The mitogenic responses of the cell mixtures to natural lipid A are shown in Fig. 6 for succinylated preparation 313. The incorporation of radioactivity was proportional to the

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**TABLE 2. Mitogenicity of synthetic lipid A analogs, lipid A, and ConA for spleen cells from C3H/HeJ mice**

<table>
<thead>
<tr>
<th>Sample</th>
<th>cpm ± SE at the following dose (μg):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>303</td>
<td>218 ± 15</td>
</tr>
<tr>
<td>316</td>
<td>237 ± 11</td>
</tr>
<tr>
<td>Lipid A</td>
<td>241 ± 4</td>
</tr>
<tr>
<td>ConA</td>
<td>3,965 ± 164</td>
</tr>
</tbody>
</table>
number of normal cells in the mixture, indicating that no suppressor cells had been formed. Similar results were obtained with succinylated preparation 302 (data not shown).

Hemolytic activity of synthesized lipid A analogs. It was found that the synthesized lipid A analogs lysed erythrocytes. The hemolytic activity was determined after incubation of sheep erythrocytes with test samples at 37°C under 10% CO₂ for different periods of time. The remaining cells were counted. Most of the synthetic samples tested showed hemolytic activity (Table 3), including natural lipid A, the latter, however, only after a long incubation period. Succinylated lipid A and lipopolysaccharide (data not shown) showed lower and no activity, respectively.

Cell toxicity of synthetic lipid A analogs for spleen cells. Since some synthetic preparations exhibited suppression of mitosis of spleen cells by lipid A as well as hemolytic activity, their possible toxic action on spleen cells was tested. Samples (100 μg/ml) of synthetic disaccharide (316, succinylated 302 and 311, and 312) and monosaccharide (succinylated 313) preparations were incubated with spleen cells for different times. Viable cells were counted using the trypan blue exclusion assay. Compared with controls, no significant decrease in viability of the cells after 24, 48, and 72 h of incubation was observed with the disaccharide preparations. In contrast, the number of viable spleen cells decreased drastically on incubation with succinylated 313, and after 24 h only 6% of the original cells were viable.

FIG. 4. Suppression by synthetic lipid A analogs of the mitogenic responses of murine spleen cells to ConA. The preparations were added to the cultures simultaneously with 0.6 μg of Con A per ml. Symbols: O, succinylated 302; ●, 303; ▼, succinylated 313; ■, 316; △, succinylated 316; Δ, 318; □, 319; x, 321.

FIG. 5. Suppression of lipid A mitogenicity by synthetic lipid A analogs (100 μg/ml) added to the cultures at different times before or after the addition of lipid A (10 μg/ml). Symbols: O, succinylated disaccharide 302; Δ, succinylated disaccharide 321; ●, succinylated monosaccharide 313; △, succinylated monosaccharide 318.

FIG. 6. Mitogenic responses to lipid A of cell mixtures containing cells pretreated with succinylated 313 and normal spleen cells in different ratios (1:0 [●], 1:1 [Δ], 1:9 [x], and 0:1 [O]). The pretreated cells had been incubated with 10 μg of succinylated 313 per ml for 3 h and washed three times with medium. Cell numbers in the mixture were adjusted to 4 × 10⁶ cells per ml.
TABLE 3. Hemolytic activity of synthetic lipid A analogs and lipid A

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Original (orig) or succinylated (succ)</th>
<th>Dose (μg/ml)</th>
<th>Hemolytic activity (%)&lt;sup&gt;a&lt;/sup&gt; after incubation for 3 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid A</td>
<td>orig</td>
<td>100</td>
<td>8.8</td>
<td>52.9</td>
<td>88.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>succ</td>
<td>100</td>
<td>0</td>
<td>17.6</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Disaccharide derivatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>303</td>
<td>orig</td>
<td>100</td>
<td>82.2</td>
<td>91.5</td>
<td>95.6</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>orig</td>
<td>100</td>
<td>86.6</td>
<td>90.8</td>
<td>92.7</td>
<td>96.9</td>
</tr>
<tr>
<td>302</td>
<td>succ</td>
<td>100</td>
<td>98.6</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>321</td>
<td>orig</td>
<td>100</td>
<td>86.8</td>
<td>91.0</td>
<td>98.4</td>
<td>100</td>
</tr>
<tr>
<td>316</td>
<td>orig</td>
<td>100</td>
<td>63.1</td>
<td>68.0</td>
<td>75.8</td>
<td>90.6</td>
</tr>
<tr>
<td>317</td>
<td>orig</td>
<td>100</td>
<td>32.0</td>
<td>42.9</td>
<td>50.3</td>
<td></td>
</tr>
<tr>
<td>311</td>
<td>succ</td>
<td>100</td>
<td>86.3</td>
<td>86.7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>312</td>
<td>succ</td>
<td>100</td>
<td>0</td>
<td>90.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>314</td>
<td>succ</td>
<td>100</td>
<td>92.7</td>
<td>92.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>315</td>
<td>succ</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<td>succ</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>318</td>
<td>orig</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>319</td>
<td>orig</td>
<td>100</td>
<td>74.6</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Structures of the synthetic preparations are given in Fig. 1 of reference 23.

<sup>b</sup> Percent of cells lysed after incubation.

**DISCUSSION**

The mitogenic effect of synthetic lipid A analogs on mouse spleen cells was studied. Some of the synthetic preparations which could not be solubilized in water were tested in their succinylated soluble form. Such a modification did not alter the pyrogenic and toxic properties of lipid A (23), but it decreased the anticomplement activity (23) and, as shown in this paper, the mitogenic activity. The results in Table 1 show that the synthetic disaccharide preparations exhibit mitogenicity to different degrees; however, compared with lipid A, their activity was lower. Although it is presently difficult to identify structures essential for mitogenicity, relatively strong activity was exhibited by preparations containing a phosphate group in position 1 of the reducing glucosamine (preparations 303 and 316) or amide-bound acyloxyacyl residues (succinylated preparations 314 and 315). A phosphate group in position 4 seemed to suppress the activity. Preparation 303, which contains as acyl residues only tetradecanoic acid and phosphate in position 1, showed the highest activity, expressing about 38% of lipid A and 100% of succinylated lipid A activity. As shown in the accompanying paper (23), preparation 303 exhibited neither pyrogenic nor lethal properties, indicating again that endotoxic activities are separable.

Neither preparation 303 nor 316 exhibited mitogenicity for spleen cells from lipopolysaccharide-nonresponder mice (strain C3H/HeJ). Furthermore, the mitogenic response to preparation 316 was abolished after complexing with polymyxin B. Both properties are characteristic for lipid A. In contrast to the disaccharides, none of the monosaccharide derivatives exhibited mitogenicity.

A number of lipid A analogs have been tested in other laboratories. To overcome difficulties regarding the solubility of the preparations, different groups of investigators have applied different methods. In the case of phosphate-containing preparations, conversion via the acidic form into the triethylammonium salt form, and in the case of other preparations, conversion into succinyl derivatives proved in our hands to be useful ways to provide soluble preparations. Other investigators have tested the synthetic preparations incorporated into liposome model membranes (15, 28) complexed with bovine serum albumin (17a) or after dispersion by ultrasonication (17). Because of the different methods of preparing the samples for the test and differences in the test systems, it is not surprising that the results obtained by the different groups show some diversity. Our data agree best with those of Kanegasaki et al. (15), Yasuda et al. (28), and Kumazawa et al. (17a), but they seemingly differ in some cases from those of Kotani et al. (17). These authors found, e.g., that preparation 313 was a fairly active mitogen, whereas in our hands this preparation could not be tested because of its insolubility; it became soluble after succinylation, but then it was highly cytotoxic. Preparation 303, which was insoluble in our hands and inactive according to Kotani et al. (17), became soluble and active when converted into the triethylammonium form. Preparation 316 was active in both studies. The significance of solubility and state of aggregation for biological activities of endotoxins has been stressed previously (6).

In the course of these investigations, some synthetic preparations were found to suppress the mitogenic response of B cells to lipid A. Optimal suppression was obtained in the case of disaccharide preparations when they were added to the spleen cells 3 h before the addition of lipid A. In contrast, the monosaccharide preparations suppressed lipid A mitogenicity at all times of addition to the system. To determine whether the synthetic preparations would suppress lipid A mitogenicity specifically, they were tested as inhibitors of the mitogenic response of T cells to ConA. It was found that the disaccharide preparations did not interfere with the ConA system; in contrast, the monosaccharide preparations did suppress ConA mitogenicity.

Further studies revealed that most of the synthetic preparations represented strong hemolysins, and one of the mono-

**TABLE 4. Characteristic properties related to mitogenicity evaluated for three synthetic preparations**

<table>
<thead>
<tr>
<th>Prepn no. and type</th>
<th>Mitogenicity</th>
<th>Suppression of mitogenicity</th>
<th>Cytotoxicity for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lipid A</td>
<td>ConA</td>
<td>Sheep erythrocytes</td>
</tr>
<tr>
<td>316, disaccharide</td>
<td>++</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>302, disaccharide</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>313, monosaccharide</td>
<td>succtinylated</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>
saccharide derivatives tested was, in addition, highly toxic for mouse spleen cells. (Lipid A also exhibits hemolytic activity but to a much lower extent than the synthetic preparations, whereas lipopolysaccharide was not lytic, in agreement with Ciznar and Shands [4], who described alkali-treated lipopolysaccharide as hemolytic.)

Table 4 summarizes the properties of three synthetic preparations which seem to be representative for three groups. Preparation 316 is one of the strongest mitogens among the synthetic preparations. It does not suppress lipid A or ConA (or its own) mitogenicity, and it is hemolytic but not toxic for spleen cells. Succinylated preparation 302 is mitogenic, it suppresses lipid A but not ConA mitogenicity, and it is hemolytic but not toxic for spleen cells.

Succinylated monosaccharide preparation 313 is not a mitogen, it suppresses both lipid A and ConA mitogenicity, and it is hemolytic and toxic for spleen cells. In this case the cytotoxic properties of the synthetic preparation are believed to prevent the expression of mitogenicity of B and T cells (preparation 316, slightly not hemolytic, has not been tested for spleen cell toxicity).

It is well known that lipopolysaccharides can enhance or inhibit immune responses, depending on the dose or timing of administration (5, 13, 18, 21, 24, 25). The mechanism by which lipopolysaccharide produces these different effects on lymphocytes is still unclear; however, recent evidence suggests that the inhibition of immune responses by lipopolysaccharides may be due to the induction of suppressor cell activity (9, 14, 16, 26, 27). The spleen cells, however, which had been made unresponsive to lipopolysaccharide stimulation by treatment with lipid A analogs, did not act as suppressor cells on normal spleen cells. The mechanisms of the suppression of lipid A mitogenicity remain unknown.

The results indicate, however, that the mono- and disaccharide preparations act in different ways. It is hoped that the synthetic lipid A analogs may serve as tools with which to study molecular events of the action of lipid A on B cells.

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LITERATURE CITED


24. Uchiyama, T., and D. M. Jacobs. 1978. Modulation of immune...