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Biological Activities of Endotoxins Detoxified by Alkylation

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It has been previously observed that lipopolysaccharides can be detoxified by alkylation and yet retain their adjuvant activity. Our present findings confirm these results and show, moreover, that these derivatives did not lose their capacity to protect mice against lethal irradiation and lost only partially their ability to interrupt pregnancy or to induce blast transformation of murine B-lymphocytes. However, in contrast with lipopolysaccharides, these alkylated preparations did not enhance the nonspecific resistance of mice to a bacterial infection. The various biological functions of endotoxins can therefore be separated and are not uniformly related to their toxicity.

It is well established that lipopolysaccharides (LPS), besides being potent immunogens, can elicit a wide range of pharmacological and immunological effects that are not related to their serospecificity. For instance, they have been shown to induce abortion (13, 20, 25), to be strong adjuvants of the humoral antibody response to a given antigen when administered simultaneously (10, 12), and to increase the host's nonspecific resistance against various infections (9, 21, 26) or against lethal irradiation (3, 28). More recently their nonspecific mitogenic activity on B-dependent lymphocytes has been demonstrated (2, 23). Since most of the studied activities could be produced by lipid A and since in most cases the preparations used were highly toxic and pyrogenic, toxicity has generally been assumed to be a prerequisite of all the biological effects of LPS (1, 15, 16, 18, 29). However, Schenck et al. have reported that derivatives of LPS detoxified by succinylation (SuLPS) maintained their adjuvant activity (27). McIntire et al. have more effectively separated adjuvant effects from toxicity by using phthalic instead of succinic anhydride. The toxicity of these sodium phthalyl lipopolysaccharides (SPLPS) was assayed by pyrogenicity in rabbits and by lethality in mice rendered susceptible to endotoxins by actinomycin D. The adjuvant effect was evaluated by the increase of the antibody titer against human serum albumin (F. C. McIntire, J. R. Schenck, and M. P. Harjie, personal communication). We have confirmed these findings by using either bovine serum albumin (BSA) or a viral vaccine as antigen and have shown in addition that, although well detoxified, SPLPS retained its mitogenic activity (7).

In the present report these previous results have been extended by measuring the activity of detoxified LPS extracted from several strains of gram-negative bacteria and by comparing in mice and in rabbits the adjuvant effect of detoxified endotoxins administered with the antigen in saline and in guinea pigs with an antigen administered in a Freund-type water-in-oil emulsion. Furthermore, our data demonstrate that after detoxification, LPS retained its capacity to stimulate murine lymphocytes, to protect mice against lethal irradiation, and to induce abortion, whereas its ability to increase nonspecific resistance to bacterial infection was abolished.

MATERIALS AND METHODS

Animals. Two-month-old mice were used. They were either Swiss common stock (C.N.R.S. Orléans Center), inbred AKR (Pasteur Institute), or (C_{57}B1 × AKR)F_1 hybrids (Pasteur Institute). In certain experiments, congenitally athymic nude mice were used. These mice homozygous for *nu* mutation were raised at the C.N.R.S. Orléans Center from an outbred hairy (*nu*/+) strain originating from the Institute of Animal Genetics (Edinburgh).

Four- to six-month-old Bouscat rabbits and male Harley guinea pigs (Pasteur Institute) weighing 350 g were also used.

LPS, SPLPS, and SuLPS preparations. LPS was extracted according to the phenol-water procedure from the following smooth strains: *Salmonella enteritidis* Danysz strain, *S. minnesota* 1111, *S. typhimurium*, and two different preparations of *S. abortus equi*, one prepared by Difco and one by Wander and referred to hereafter as Pyrexal. LPS (glycolipid) extracted from a heptoseless Re mutant of *Escherichia coli* and given to us by Sheldon Wolff was also tested. These LPS preparations were detoxified as follows: 100 mg of LPS and 1 g of phthalic anhydride or succinic anhydride were dissolved in 5 ml of formamide and 5 ml of pyridine. The preparation was the same as that described previously with succinic anhydride (27). The alkylated LPS contained approximately equal weights of succinyl or

phthalyl and LPS. In certain experiments SPLPS originating from *E. coli* and prepared by F. C. McIntire was tested in comparison. Lipid A was prepared by C. Galanos according to a procedure previously described (15).

Antiserum. Antiserum was prepared in rabbits by intravenous injections (four per week during 1 month) of alum-adsorbed *S. enteritidis* endotoxin. Each injection was made with 5 μg of endotoxin. Animals were bled 6 days after the last injection.

Antigens. In experiments in which the adjuvant activity was measured, the antigens used were the following: BSA (fraction V; Miles), ovalbumin (hen egg albumin six times crystallized; Miles) and influenza vaccine (Mutagrip, Institut Pasteur).

Modification of antigenicity. To test the effect of alkylation on the antigenic determinants of LPS, double-diffusion experiments were performed by the method of Ouchterlony in special agar Noble (Difco) (1% in 0.025 M borate buffer, 0.08 M NaCl). Serospecificity was also detected by inhibition of passive hemagglutination.

Toxicity. The lethal potency of the various samples was measured by intravenous injection in mice that had been adrenalectomized 48 h previously (8). They were challenged with threefold dilutions of various preparations suspended in saline. At least eight mice were used at each dosage level. No deaths occurred later than 2 days after challenge and were therefore recorded during 48 h. The mean lethal dose was calculated according to the method of Reed and Muench (24).

Abortion. Swiss mice between the 16th and 18th days of pregnancy were used in all experiments. Females were selected by palpation, 2 weeks after having been isolated from males with which they had consorted for 3 days. Under these conditions no normal background rate of abortion was observed after injecting pyrogen-free saline to 17 mice. The abortive effect of the compounds injected intravenously in a volume of 0.2 ml was controlled by sacrificing the mother 24 h later. The survival of the fetuses was checked by their mobility and heart beats.

Adjuvant activity. Mice were immunized either subcutaneously with 0.5 mg of BSA or intraperitoneally with 50 U of influenza vaccine. Rabbits were immunized intravenously with several injections of 2 mg of BSA. The antigens were injected in saline with or without various dosages of the adjuvant preparations. All animals were boosted by a second injection of antigen in saline without adjuvant.

Guinea pigs were immunized with 1 mg of ovalbumin administered with or without the endotoxin preparations in a water-in-oil emulsion with Freund incomplete adjuvant (FIA). The mixture was injected in both hind footpads and the guinea pigs were bled 21 days later after immunization.

Antibody estimation: passive hemagglutination. Formalinized sheep erythrocytes were coated according to procedures previously described, either with endotoxin (14), BSA, or ovalbumin (11). All tests were performed under conditions previously described. Agglutination titers were expressed in terms of serum dilution (reciprocal titer), whereas the quantity required for inhibition represented the absolute amount of antigen deposited in each cup (8).

Hemagglutination inhibition of chicken erythrocytes. Titrations of the pooled sera of mice immunized with influenza virus were performed according to the method prescribed by the W.H.O. Committee (30). Titers were expressed as the reciprocals of the highest dilution by which hemagglutination was completely inhibited.

Antibody titers to BSA. Antibody titers to BSA were also evaluated by measuring the antigen-binding capacity of the serum with [^{125}I]BSA according to the procedure of Minden and Farr (33% of the antigen added) (17). The antibody titers to ovalbumin were also evaluated by quantitative precipitation. Skin tests were performed by intradermal injection of 1, 5, or 10 μg of ovalbumin. Animals were observed at various time intervals during 48 h, at which time positive responses were considered to be of delayed hypersensitivity type.

Blast transformation. The blast transformation of lymphocytes was determined by the scintillation method. Eighteen hours before harvest, 1.5×10^6 spleen cells were incubated with 1.5 μCi of [^{3}H]thymidine (specific activity, 1 Ci/mmol) (Saclay, France). The processing of [^{3}H]thymidine-treated culture was described previously. The details of this procedure were essentially the same with the various lymphocytes, although the conditions of the lymphocyte culture vary according to the origin of the cells (5).

***Klebsiella* infection.** In certain experiments, mice were challenged intravenously with 5×10^5 *K. pneumoniae* type II. Certain groups were stimulated by the same route with various endotoxic preparations. Protection was measured by survival time.

Irradiation. Protection by LPS and its alkylated derivatives was measured by injecting the preparations intraperitoneally 24 h before irradiation. Whole-body irradiation was delivered from a cobalt-60 source (type Lysa III, Picker International Corp.), which at the time of the study was delivering approximately 100 rpm. Mice were confined singly in a lucite container and exposed under identical conditions. The dose received (950 R) represents a 100% lethal dose for (C_{57}B1 × AKR)F_1 hybrids. Irradiated mice received 500 U of dihydrostreptomycin-bipenicillin subcutaneously 24 h after irradiation.

RESULTS

Toxicity. In the following experiments, mice were rendered susceptible to LPS by adrenalectomy 48 h before challenge. Five different endotoxin preparations and their detoxified derivatives, plus the SPLPS prepared by McIntire (*E. coli* K235) and the SPLPS prepared from the Re mutant of *E. coli*, were injected intravenously at various dosages, using for each dose a group of eight mice. Whereas the mean lethal dose of the toxic antigens varied between 0.037 and 0.21 μg, none of the SPLPS preparations killed 50% of the mice at 300 μg, which was the highest dose assayed. A comparable detoxification was also observed with phthalylated LPS from

the heptoseless Re mutant. Thus, the preparations were at least 10,000 times less toxic than the LPS from which they had been prepared (Table 1). It must be added that storage in saline at 4 C for 3 months did not restore toxicity of the two SPLPS preparations tested (*S. enteritidis* and *E. coli* K235). The LPS was well detoxified by succinylation, although to a lesser degree than by phthalylation (Table 1).

Endotoxin-induced abortion. In previous experiments it was shown that abortion could be induced by administering 1 to 10 μg of *S. enteritidis* LPS (20). Therefore, 0.1, 1, 3, or 10 μg of this LPS preparation was administered intravenously to pregnant mice. Other groups received phthalylated derivatives of LPS extracted from *S. enteritidis* or *E. coli* K235 at dosages varying between 10 and 100 μg. The effect on pregnancy of 100 μg of SPLPS was comparable to that produced by 1 μg of LPS (Table 2), although this dose of SPLPS represented in toxicity less than 0.01 μg of LPS (see Table 1).

Modification of antigenicity by alkylation. The antigenicity of LPS and SPLPS extracted from *S. enteritidis* was tested by inhibition of passive hemagglutination of endotoxin-sensitized sheep erythrocytes. Whereas 0.06 μg of LPS was sufficient to inhibit agglutination completely, only a weak inhibition was obtained with 100 μg of SPLPS. This serum, which was prepared by immunizing a rabbit with *S. enteritidis* LPS and was used undiluted in the following gel diffusion experiments, had a titer of 1:800 (as measured by passive hemagglutination). Fifty micrograms of each preparation was deposited in the wells. A precipitating band was obtained against the wells containing *S. enteritidis* LPS but not against the well containing *S. abortus equi* (Fig. 1). No precipitating band could be observed when the wells contained SPLPS of *S. abortus equi* or even SPLPS of *S. enteritidis*. Similar negative results were observed when SuLPS of *S. enteritidis* was used with *S. enteritidis* antiserum.

TABLE 1. *Toxicity in adrenalectomized Swiss mice*

LPS	Mean lethal dose (μg)
S. enteritidis (Danysz strain)	
LPS	0.037
SPLPS	>300
SuLPS	30
S. typhimurium	
LPS	0.044
SPLPS	>300
S. minnesota 1111	
LPS	0.086
SPLPS	>100
S. abortus equi (Difco)	
LPS	0.21
SPLPS	>300
S. abortus equi (Pyrexal)	
LPS	0.05
SPLPS	>300
E. coli O26:B6 LPS	0.063
E. coli K235 SPLPS	>300
E. coli (Re strain)	
LPS	0.055
SPLPS	>300

TABLE 2. *Endotoxin-induced abortion in Swiss mice*

Treatment	Dose (μg)	Abortion[a]	Percentage of dead fetuses
S. enteritidis LPS	0.1	3/10	40
	1	7/10	50
	3	10/10	68
	10	10/10	100
S. enteritidis SPLPS	10	0/5	0
	30	1/4	38
	100	3/4	60
E. coli K235 SPLPS	30	1/7	2
	100	8/8	52

[a] Number of abortions/total checked after 24 h.

Adjuvant activity of alkylated endotoxins. (i) Activity of SPLPS and SuLPS administered with BSA in saline to mice. The enhancement of circulating antibodies was measured by administering either LPS (*S. enteritidis*), SPLPS from *S. enteritidis*, or SPLPS from *E. coli* with BSA at various dosages to groups of 10 mice. Whereas the LPS was administered at a dose of 30 or 100 μg, the alkylated preparations were administered at doses varying between 30 and 300 μg. Higher doses of unmodified LPS could not be used because of lethal effects. The mice were bled at various intervals before and after a secondary response, and the titer was evaluated either by passive hemagglutination or by antigen-binding capacity of the sera.

A marked adjuvant effect was observed when the antigen was administered with endotoxin or with the SPLPS preparations (Fig. 2). The same order of magnitude was obtained by injecting only a threefold amount of SPLPS which contained, as previously stated, 50% sodium phthalyl and was at least 10,000 times less toxic than LPS.

In a similar experiment, in which SuLPS was used only at the dosage of 100 μg, a 10-fold increase in antibody titer was observed in comparison with the controls that had received only BSA. It must be noted that SuLPS, although more toxic than the phthalylated derivative,

had an adjuvant effect that seemed less marked.

(ii) Activity of SPLPS administered with influenza vaccine in saline to mice. Fifty units of influenza vaccine was administered with or without 100 μg of SPLPS. A third group of controls received only an injection of saline to evaluate spontaneous background activity. The animals were bled at different time intervals and their antibody titer was measured by inhibition of hemagglutination. A marked increase of the antibody titer was obtained when the vaccine was administered with SPLPS (Table 3).

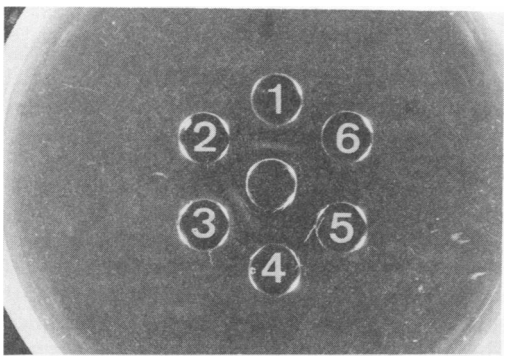

FIG. 1. *Precipitation pattern in agar or LPS and SPLPS with undiluted anti-S. enteritidis rabbit serum (1 and 3), S. enteritidis LPS (2 and 4), S. enteritidis SPLPS (5), S. abortus equi LPS (6), S. abortus equi SPLPS.*

(iii) Activity of SPLPS administered with BSA in saline to rabbits. Rabbits received on days 1, 4, and 7 intravenous injections of 2 mg of BSA in 0.5 ml of saline with or without the adjuvants. A recall of 2 mg of BSA without adjuvant was made by the same route on day 40. No detectable antibody titers were found in the controls (Table 4). On day 14, the two rabbits treated with 10 μg of LPS and three rabbits out of four treated with 100 μg of SPLPS showed high titers. All animals treated with adjuvants showed high titers after the recall, whereas the two controls had no detectable titers.

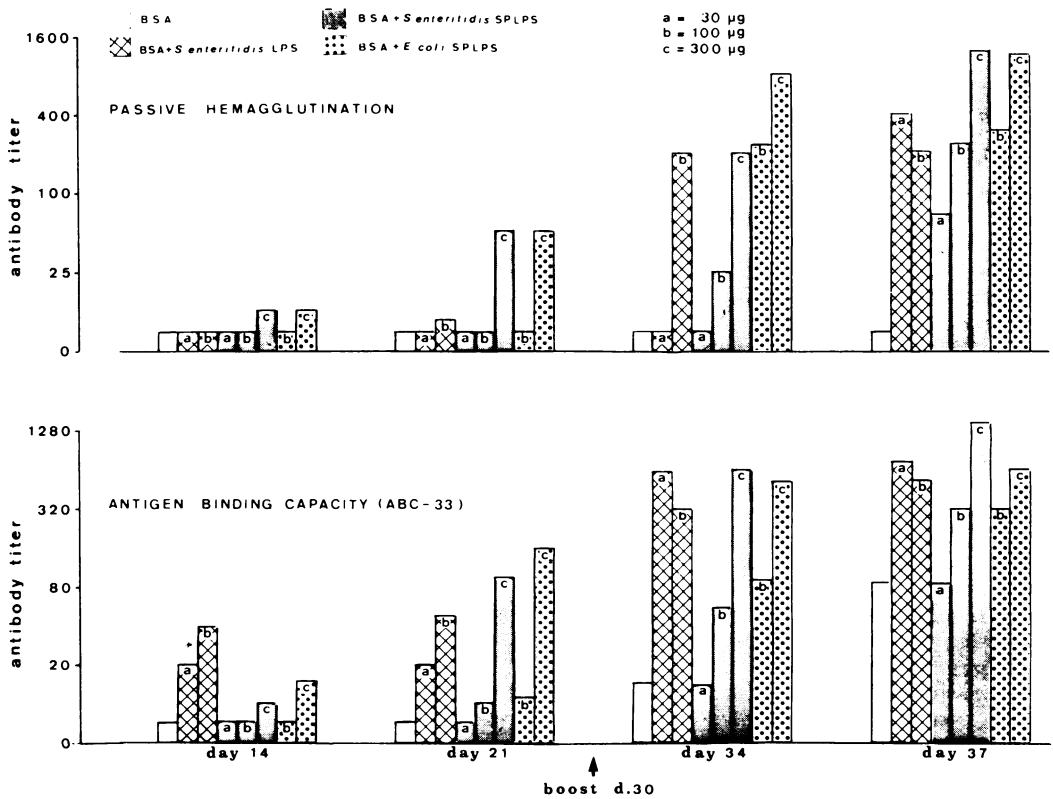

FIG. 2. *Antibody response of Swiss mice to BSA in saline. Ten mice were used per group. They received subcutaneously 500 μg of BSA with or without adjuvant in 0.5 ml of saline. Thirty days later, they received under the same conditions 100 μg of the antigen alone as a boost. Values at days 14, 21, 28, and 34 were made from pooled sera. Titers given at day 37 express the arithmetic mean of the separate evaluation.*

TABLE 3. *Influence of SPLPS on the antibody response of mice immunized against influenza virus*[a]

Treatment	Antibody titer (hemagglutination inhibition)				
	Day 14	Day 21	Day 28	Day 35	Day 42
Control	<12.5	<12.5	<12.5	<12.5	<12.5
Influenza vaccine (50 U)	25	50	50	12.5	25
Influenza vaccine (50 U) + *S. enteritidis* SPLPS (100 μg)	25	200	200	100	100

[a] Swiss mice received all preparations intraperitoneally in 0.2 ml. A boost of 50 U of vaccine alone was done at day 15.

TABLE 4. *Antibody response of rabbits immunized by BSA with LPS and SPLPS in saline*[a]

Adjuvant	Antibody titers (passive hemagglutination)			
	Day 14	Day 44	Day 47	Day 60
Controls	1	1	<1	<1
	1	1	<1	<1
S. enteritidis	400	200	800	400
LPS (10 μg)	200	100	400	50
S. enteritidis	3	100	800	50
SPLPS (100 μg)	50	2	100	12.5
	50	400	800	200
	50	25	200	25

[a] At days 1, 4, and 7, each animal received intravenously 2 mg of BSA with the appropriate adjuvant in 0.5 ml of saline and at day 40 a recall of 2 mg of BSA alone by the same route.

(iv) Activity of SPLPS administered with ovalbumin in FIA to guinea pigs. In these experiments, 3 mg of SPLPS was administered on a weight basis whereas, because of its toxicity, only 500 μg of LPS was administered with ovalbumin in a water-in-oil emulsion using FIA. The controls received either FIA or Freund complete adjuvant with the antigen.

As shown in Table 5, 0.5 mg of LPS gave an increase in humoral response as measured by precipitins or passive hemagglutination, but no delayed hypersensitivity. In contrast, 3 mg of SPLPS gave a more marked increase in circulating antibodies. Positive skin test reactions to ovalbumin were observed, although these responses were weaker and more transient than those observed with Freund complete adjuvant.

Mitogenicity of SPLPS. It was previously reported that SPLPS stimulated the spleen lymphocytes of AKR or nude mice and that there was a dose-response relationship. Although phthalyl LPS was active at greater dosages, its mitogenicity could not be accounted for by the presence of residual toxic molecules since the toxicity detectable in SPLPS was at least 10,000 times smaller (7).

Since it has been established that the mitogenicity of LPS is related to lipid A, which represents the molecule toxic moiety, 50 μg of SPLPS was tested in comparison with either 50 μg of LPS or 10 μg of lipid A and incubated with spleen lymphocytes of AKR or nude mice. These dosages were chosen because they were shown to be optimal in preliminary experiments. A strong stimulation was observed both with toxic preparations and with the nontoxic SPLPS (Table 6).

In the following experiments, 50 μg of either *S. minnesota* LPS or *S. enteritidis* SPLPS was tested with human peripheral lymphocytes or with monkey, rabbit, and murine spleen suspensions. Both preparations markedly stimulated the incorporation of thymidine by nude mice spleen lymphocytes, whereas they had no significant effect on lymphocytes of the other species (Table 7). These results confirm that like LPS, its detoxified derivative is a mitogen of murine B-derived lymphocytes.

Influence of LPS, SPLPS, and SuLPS on nonspecific resistance to a bacterial infection. In these experiments, mice received LPS and SPLPS at various dosages intravenously 24

TABLE 5. *Adjuvant effect of SPLPS in guinea pigs immunized by ovalbumin*

Treatment[a]	Passive hemagglutination[b]	Precipitins (μg of N/ml)	Skin test[c] (diam in mm)
FCA	1,200	5,700	10 + necrosis
FIA	500	250	Negative
FIA + LPS (0.5 mg)	2,500	1,200	Negative
FIA + SPLPS (3 mg)	1,600	3,840	5

[a] Each animal (six per group) received 1 mg of ovalbumin emulsified in FIA or Freund complete adjuvant (FCA). LPS and SPLPS were prepared from *S. enteritidis*.

[b] Averages expressed as reciprocal titer of serum.

[c] Averages expressed 48 h after injection.

TABLE 6. *Blastogenic reactivity to endotoxin preparations in mouse spleen lymphocytes*

Mitogen	Index[a] in nude mouse						Index[a] in AKR mouse		
	1[b]	2	3	4	5	Mean	1	2	Mean
S. minnesota LPS (50 μg)	10.6	27.7	9.8	3.8	6.4	11.6	14.9	11.2	13.05
S. minnesota lipid A (10 μg)	4.5	12.3	6.7	4.3	7.4	7.04	5.7	8.5	7.1
S. enteritidis SPLPS (50 μg)	7	17.1	6.8	3.3	3.7	7.58	4.7	5.5	5.1

[a] Stimulation index = radioactivity of treated lymphocytes/radioactivity of control lymphocytes. The means represent the averages of experiments made in triplicate determinations.
[b] Number of mice.

TABLE 7. *Blastogenic reactivity to endotoxin preparations in different species*

Mitogen	Human blood lymphocytes	Monkey spleen lymphocytes	Rabbit spleen lymphocytes	Nude mouse spleen lymphocytes
S. minnesota LPS	2.26 ± 1.36[a]	1.33 ± 0.77	2.66 ± 1.84	10.2 ± 0.32
S. enteritidis SPLPS	1.03 ± 0.3	1.17 ± 0.44	1.05 ± 0.33	6.9 ± 0.6

[a] Mean of stimulation index of five subjects ± standard deviation. Stimulation index = radioactivity of treated lymphocytes/radioactivity of control lymphocytes.

h before being challenged with 5×10^5 bacteria. Under these severe conditions, all the controls died in less than 24 h (Table 8). Animals treated with LPS were very effectively protected, since 0.01 μg was active and seven mice out of eight survived after injection of 1 μg. In contrast, SPLPS even when injected at the high dosage of 100 μg or SuLPS at 10 μg was ineffective. The slight activity obtained with 1 mg of SPLPS can be attributed to some residual toxicity since the effect was comparable to that obtained with 0.01 μg of the toxic antigen (Table 8). In view of these findings, larger amounts of SuLPS were not assayed.

Protection against irradiation. In the following experiments LPS, SuLPS, or SPLPS was injected to groups of 20 $(C_{57}B1 \times AKR)F_1$ hybrids. The protective effect of LPS and its detoxified derivatives against lethal irradiation by 950 R was measured by evaluating the number of survivors, deaths being recorded between 8 and 30 days. Whereas all the untreated controls died before 30 days, 100% of the animals treated by LPS and 75% of those treated by SuLPS survived (Fig. 3a). This protection was highly significant in both cases. When phthalylated LPS was used, the protection was weaker although still statistically significant (Fig. 3b).

The protective effect of SPLPS was confirmed in an experiment in which Swiss mice were used. These mice were more resistant against the same irradiation dose, and only 40% of the controls died. Under the same conditions no death was recorded in the groups treated either by LPS or by SPLPS. These differences were highly significant (Fig. 4).

TABLE 8. *Influence of LPS and alkylated derivatives on nonspecific resistance of Swiss mice infected with K. pneumoniae*

Treatment[a] (μg)	MST[b]	Survival at day 15 after challenge
Control (saline)	1	0/8
S. enteritidis LPS		
0.01	2	3/8
0.1	>15	5/8
1	>15	7/8
S. enteritidis SPLPS		
1	1	0/8
10	1	0/8
100	1	0/8
1,000	3	2/8
Control (saline)	1	0/6
S. enteritidis LPS		
0.001	1	0/6
0.01	5	1/6
0.1	6	3/6
S. enteritidis SuLPS		
0.1	1	0/6
1	1	0/6
10	3	0/6

[a] Twenty-four hours before infection.
[b] MST, median survival time, i.e., day at which 50% of mice were dead.

Although SuLPS appeared to be more active than SPLPS, its effect cannot be explained by its residual toxicity since the amount used (30 μg) corresponded to 0.03 μg of LPS in adrenalectomized mice. Such a small amount of endotoxin has no protective effect against 950 R.

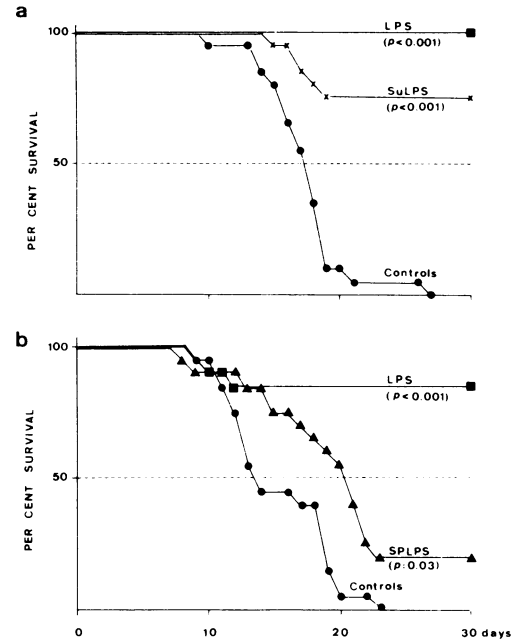

FIG. 3. *Protection against lethal irradiation in* ($C_{57}Bl \times AKR)F_1$ *hybrids with 30 μg of S. enteritidis LPS, SuLPS, or SPLPS. Significance between each treated group and controls was calculated by Mann and Whitney's test.*

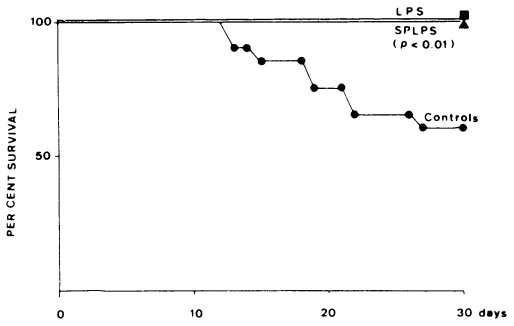

FIG. 4. *Protection against irradiation in Swiss mice with 30 μg of S. enteritidis LPS or SPLPS. Significance between each treated group and controls was calculated by Mann and Whitney's test.*

DISCUSSION

Using a very sensitive assay, the data reported here confirm that the sodium phthalate derivatives of several endotoxin preparations were at least 10,000 times less toxic than LPS, whereas SuLPS was approximately only 1,000 times less toxic. Moreover, the antigenic determinants of both derivatives were modified after these procedures. Nevertheless, as was previously demonstrated (27), these derivatives enhanced markedly the humoral antibody response to various antigens after administration to mice, rabbits, and guinea pigs. The nontoxic SPLPS also retained its ability to induce blastic transformation of mouse spleen B-dependent lymphocytes (7). Although the response observed required higher dosages than required with LPS, this effect was not related to the presence of residual toxic antigen in the preparation, as was demonstrated by comparative evaluation of toxicity. The decreased mitogenic response in other species (Table 7) could perhaps be accounted for by the loss of antigenicity of the molecule. It has indeed been reported that LPS is apparently a nonspecific mitogen only in small rodents but that its polysaccharidic hapten can also induce a slight stimulation of lymphocytes (4).

It has been well established that very small amounts of endotoxin can protect mice against a virulent strain of *Klebsiella* (19, 21) even if these organisms have been rendered resistant to antibiotics by mutation or by plasmid transfer (22). In contrast, although their adjuvant activity had been retained, the detoxified molecules had lost their capacity to protect mice against a *Klebsiella* infection. Such results indicate, at least in this experimental system, that enhancement of nonspecific resistance to infection by endotoxins is not related to their adjuvant or mitogenic activities.

In contrast to the loss of their capacity to increase resistance to infection, the nontoxic alkylated LPS preparations maintained their ability to protect mice against lethal irradiation. This effect of LPS has been related to its capacity for stimulating bone marrow stem cells. It has recently been reported that nonendotoxic breakdown products of bacterial LPS could release colony-stimulating factor whereas lipid A was significantly less active than LPS or these polysaccharide preparations (6). Nevertheless, it must be noted that SuLPS, like SPLPS, have lost both their toxicity and their polysaccharidic serospecificity.

Our present findings show that several but not all biological activities could be maintained in detoxified preparations of LPS. Furthermore, the degree of detoxification varied according to the assay used. Thus, as compared with LPS, a 100-fold larger dose of SPLPS was sufficient to induce abortion, whereas the difference was at least of the order of 10,000 when adrenalectomized mice were used to measure toxicity.

Therefore, the various responses elicited by this unique antigen were not uniformly and strictly correlated with its toxicity as has been generally assumed. Further investigations of the biological activities of nontoxic endotoxin derivatives should lead to a better understand-

ing of the relationships between activity and structure of LPS. Such studies may also pave the way for applications of this extremely potent bacterial immunostimulant.

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