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Interactions of the Complement System with Native and Chemically Modified Endotoxins

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Endotoxic lipopolysaccharides (LPS) isolated from *Serratia marcescens*, *Veillonella alcalescens*, and *Salmonella typhosa* were potent in their ability to induce fixation of complement (C′) in normal guinea pig, rabbit, mouse, and human serum. The C′-fixing ability of LPS was pronounced even when assays were performed in undiluted serum, and was lost after each of four chemical modifications which resulted in loss of biological toxicities. The detoxification procedures had in common the cleavage of ester-bound, long-chain carboxylic acids. The ability of biologically active LPS to fix C′ in *normal* guinea pig serum was reflected chiefly in dramatic uptake of classical C′3 (C′3t); fixation of C′1, C′4, and C′2 was virtually undetectable. Hence, it was the capacity for fixation of C′3t which was lost most overtly during detoxification. Addition of immune serum to the assay mixtures resulted in detectable fixation of C′1 and C′4. Biologically active LPS also fixed more of these components than did detoxified LPS. Immune serum restored the ability of detoxified LPS to fix C′3t, but whether this is by the original pathway is not yet clear. We concluded that the loss of certain biological activities and the loss of ability to fix C′3t in normal serum after LPS detoxification involved loss or rearrangement of substrates on LPS which either initiated or supported, or both, its interaction with the complement system. It was apparent that the ability to fix C′ can serve as a valuable in vitro indicator of the integrity of the toxic conformation of biologically active LPS membrane fragments. These experiments supported the hypothesis that certain of the biological activities induced by endotoxins are mediated via the complement system.

Recent investigations have shown that, in the interaction between bacterial lipopolysaccharides (LPS) and the complement (C′) system, characteristic C′-mediated lesions are formed on the LPS surface concurrent with loss of hemolytic C′ activity in the reaction mixture (1; J. H. Humphrey, R. R. Dourmashkin, and S. N. Payne, Immunopathology Conference, 4th, Punta Alla, 1967, *in press*.). Since lesion formation requires the participation of the terminal C′ component (U. Hadding, H. J. Müller-Eberhard, and A. P. Dalmasso, Federation Proc. **25**:485, 1966), and since various biologically active factors are generated by the C′ system in its sequential interactions prior to terminal component activation (6, 14, 48), it was suggested that certain of the biological effects of endotoxins might be mediated via the C′ system (1).

LPS is derived from the outer cell membrane (2) and is itself a membranous structure (1, 5, 38). Further, it represents a relatively stable product which is partially purified from the remainder of the cell. Therefore, it was thought that a study of LPS-C′ interactions would yield certain insights into the general chemistry and biology of C′-membrane interactions.

In the experiments reported, we have investigated interactions of C′ with so-called "endotoxoids," endotoxins pretreated chemically so that, while their antigenic capacity is maintained, their toxicity is diminshed (8, 15, 27–30, 46, 47). This report summarizes experiments initially designed to determine whether the C′-fixing properties of endotoxoids also are diminished or otherwise altered.

MATERIALS AND METHODS

Endotoxic LPS. The LPS used in most of the experiments was derived from *Serratia marcescens* by the trichloroacetic acid extraction procedure as previously

described (32). The final preparation, in the lyophilized state, is designated "parent material." LPS also was derived from *Veillonella alcalescens* strain V5 and from *Salmonella typhosa* strain 0901 by the phenol water extraction procedure of Westphal and Lüderitz (51). Interactions of the *V. alcalescens* LPS and the C′ system have previously been described (1).

Endotoxoids from S. marcescens LPS. Detoxification of the parent material, which yielded endotoxoids, was accomplished by four different procedures described earlier (29, 47). Endotoxoid-1 was obtained by transesterification; the parent material was refluxed with 2% boron trifluoride in anhydrous methanol. Endotoxoid-2-A and endotoxoid-2-B were prepared by deacylation with 0.02 M and 0.10 M sodium methylate in anhydrous methanol, respectively. Insoluble residues were filtered, washed with methanol, and then vacuum-dried. Endotoxoid-3 was obtained by the heating of the parent material at 100 C in concentrated pyridine and formic acid (2:1). Endotoxoid-4 was prepared by incubation of the parent material in 0.1 N sodium hydroxide for 24 hr at room temperature, followed by dialysis against multiple changes of distilled water. The chemistry of these reactions has been discussed (47).

Zymosan. Zymosan was obtained from General Biochemicals Corp., Chagrin Falls, Ohio.

Sheep erythrocyte stroma. Stroma was prepared from washed sheep erythrocytes according to the procedure of Mayer (18). The final preparation was suspended in distilled water and then lyophilized.

Aggregated human γ-globulins (AHGG). AHGG were prepared by the heating of the Cohn Fraction II of human serum (Hyland Laboratories, Los Angeles, Calif.) for 20 min at 63 C.

Anti-S. marcescens serum. This antiserum was prepared by hyperimmunization of adult New Zealand White rabbits with saline-washed, heat-killed organisms.

Sources of various mammalian sera. Guinea pig serum was obtained from Texas Biologicals, Inc., Fort Worth, Tex. Normal human serum was provided by young-adult male laboratory personnel. Rabbit serum was collected from New Zealand White rabbits (2 kg) obtained from the National Institutes of Health. Mouse serum was derived from 8-month-old males, Balb/c strain, of the National Institutes of Health. All sera were maintained at −70 C prior to assays.

C′ fixation. The C′-fixing activity of the LPS preparations was quantitated both in diluted and undiluted serum assay systems. In the initial experiments, a dilution of guinea pig serum in 0.5 ml was incubated with 0.4 ml of buffer or antiserum dilution and 0.1 ml of LPS suspension for 1 hr at 37 or 0 C. Residual total C′ hemolytic activity was measured by the method of Osler et al. (34). Residual classical C′ components were assayed with intermediates (18, 26) as previously described (1, 9, 10). The C′-fixing ability of LPS was also examined in 1.0-ml samples of reaction mixtures which contained progressively greater amounts of guinea pig serum (0.01 to 0.90 ml). Finally, the C′-fixing ability of these preparations was tested in undiluted serum of mammalian species whose C′ systems (guinea pig, human) or whose responses to endotoxins (rabbit, mouse) have been best defined. In the assay with guinea pig or human serum, 0.1 ml which contained the desired concentrations of LPS was added to 1.0 ml of undiluted serum. The mixture was incubated at 37 C for 1 hr, and the amount of C′ which was fixed was determined by the method of Osler et al. (34). Reaction of LPS with undiluted rabbit serum was tested in the same way, except that, because of the lower hemolytic C′ activity of rabbit serum in this system, 5×10^7 rather than the conventional 5×10^8 sensitized-indicator sheep cells were used. In mouse serum, hemolytic C′ activity against sensitized sheep cells was present in still lower titer, and it had decreased stability during preincubation at 37 C. Thus, the LPS-C′ interaction was tested by adding 0.1-ml samples of the desired concentration of LPS to 0.5 ml of mouse serum. This was preincubated for only 30 min at 37 C, and the amount of C′ which was fixed was determined by the assay of Terry et al. (45).

Lethal activity in mice. Mouse lethality was tested by intraperitoneal injection of groups of 5 to 10 adult Swiss Webster mice with graded doses of the LPS preparations. Deaths were scored at 24, 48, and 72 hr. These tests served to confirm reduction in lethality of the parent material after the detoxification procedures. Other biological effects of these preparations have been extensively reported elsewhere (15, 29, 47).

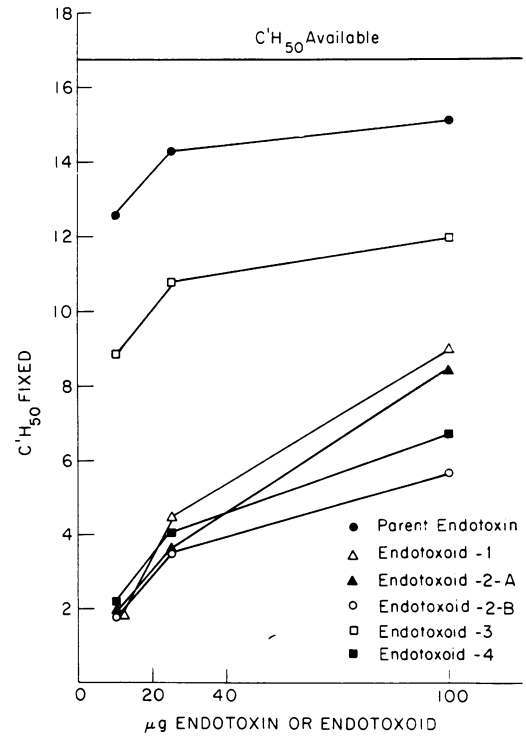

FIG. 1. *Ability of Serratia marcescens endotoxin and endotoxoids to fix total hemolytic C′ activity in guinea pig serum during 1 hr of preincubation at 37 C.*

RESULTS

Fixation of hemolytic C′ and C′ components in diluted guinea pig serum at 37 C. The ability of the parent material to fix total hemolytic C′ activity during a 1-hr preincubation period at 37 C was compared with the C′ fixing ability of five derivative endotoxoids (Fig. 1). In this experiment, the several preparations were reacted with 0.1 ml of guinea pig serum in a total volume of 1.0 ml. The parent material fixed more C′ than did any of the detoxified derivatives. The contrast was most apparent at the lowest dosage tested (10 μg), since the amount of C′ fixed by larger amounts of the parent LPS reached a maximum which was limited by the amount of C′ available.

The C′-fixing capability of an LPS preparation correlated well with its mouse-lethality activity (Table 1). It is interesting that the only endotoxoid to show a significant degree of C′-fixing capacity (endotoxoid-3) was the preparation which was the least detoxified on the basis of mouse lethality (Table 1), rabbit lethality (15), and rabbit pyrogenicity (15).

The fixation of hemolytic C′ component activities by the parent material and endotoxoid-2-A was compared during incubation at 37 C with normal guinea pig serum. It was found (Table 2) that the previously described C′-fixing capacity of the parent material could be attributed entirely to fixation of C′3t (the classical C′3 component now known to include the C′3, C′5, C′6, C′7, C′8, and C′9 of current terminology). Both the

TABLE 1. *C′-fixing capacity of Serratia marcescens endotoxin and five detoxified derivatives*

Prepn	Method of detoxification	Mouse lethality (μg for LD_{50})	$C'H_{50}$ "fixed" by 10 μg during 1 hr at 37 C (no supplementary immune serum)[a]	$C'H_{50}$ "fixed" by 10 μg during 1 hr at 0 C[b]			
				Dilution of supplementary antiserum			
				0[c]	1:200	1:100	1:50
Parent endotoxin	None	350	12.6	<1.0	4.9	7.6	15.5
Endotoxoid-3	Pyridinium	2,000	8.8	<1.0	<1.0	3.4	7.9
Endotoxoid-2-A	Sodium methylate (0.02 N)	3,000	2.0	<1.0	<1.0	3.7	17.7
Endotoxoid-4	Sodium hydroxide	4,000	2.0	<1.0	<1.0	3.8	15.4
Endotoxoid-2-B	Sodium methylate (0.10 N)	>4,000	2.0	<1.0	<1.0	3.2	12.7
Endotoxoid-1	Boron trifluoride	>4,000	1.9	<1.0	<1.0	<1.0	2.9

[a] 5 $C'H_{50}$ available.
[b] 20 $C'H_{50}$ available.
[c] No antiserum added.

TABLE 2. *Effect of rabbit anti-Serratia marcescens serum upon fixation of guinea pig complement by S. marcescens endotoxin and endotoxoid at 37 C (C′ fixation expressed in 50% units)*[a]

Material tested	Antiserum dilution	C′	C′1	C′4	C′2	C′3t
Parent endotoxin	0[b]	10.9	1,000	<500	<350	80.9
	1:200	—	5,190	3,450	—	—
	1:100	>13.1	6,000	>4,200	2,100	74.7
	1:50	—	>6,000	>4,200	—	—
	1:10	>13.1	>6,000	>4,200	2,000	81.6
Endotoxoid-2-A	0[b]	1.7	<1,000	<500	<350	7.3
	1:200	—	3,225	3,150	—	—
	1:100	3.8	5,250	>4,200	1,075	43.0
	1:50	—	6,000	>4,200	—	—
	1:10	>13.1	>6,000	4,200	1,975	79.1
Activity available	—	14.0	6,300	4,500	4,500	91.5

[a] *S. marcescens* endotoxin or endotoxoid (10 μg) was incubated with approximately 14 $C'H_{50}$ guinea pig C′ in 1.0 ml of buffered saline at 37 C for 1 hr. Varying amounts of rabbit anti-*S. marcescens* serum were added. Residual C′ and C′ component hemolytic activities were measured by assay with intermediates.
[b] No antiserum added.

parent material and the detoxified derivative failed to induce significant fixation of C'1, C'4, or C'2 in the assay systems employed. Therefore, it was the loss of ability to induce fixation of C'3t in normal guinea pig serum which was most apparent after the detoxifixation procedures.

The addition of rabbit anti-*S. marcescens* serum to the reaction mixtures restored the capacity of the endotoxoid to fix C'3t. Indeed, in the presence of large amounts of antiserum, the detoxified derivative could fix as much C'3t as could the maximally reacting parent material (Table 2). However, this addition of antibody led to marked fixation of C'1, C'4, and C'2 by each of the LPS preparations. Therefore, fixation of large amounts of C'3t with minimal fixation of the early-acting components remained characteristic only of the unmodified LPS which interacted with normal serum.

It is not yet clear whether different sites on LPS are responsible for the initiation of the interaction of LPS with C' in the presence and absence of immune serum. Attention here is directed to the capacity of the unmodified LPS to react with normal guinea pig serum in a way which seems to lead to selective fixation of C'3t, an ability which is lost during the detoxification procedures.

Fixation of hemolytic C' and C' components in diluted guinea pig serum at 0 C. C' fixation by the LPS derivatives was also tested in the presence of rabbit anti-*S. marcescens* serum during a 1-hr preincubation period at 0 C. At this temperature, virtually no C' activity is removed from guinea pig serum by endotoxic LPS unless immune serum is added. If immune serum is added, then C' uptake may depend largely upon fixation of the early-acting C' components (1, 19, 33). Therefore, it was thought that assay at 0 C might serve as an indicator of the integrity of the haptenic or C'-binding material, or both, on the LPS preparations involved in substrate-(antibody)-C' interactions which precede the C'3t steps.

We found that 10 μg of each of the LPS preparations could react with antibody to result in C' fixation (Table 1). With large amounts of antibody (e.g., 1:50), near-maximal C' fixation was achieved by detoxified as well as the active LPS preparations, but assays with graded amounts of antibody proved that the parent material was more effective.

Studies of the C' components during these reactions revealed that no detectable fixation of C'2 or C'3t had occurred. As expected, fixation of C'1 and C'4 accounted for all of the uptake of total C' (Table 3). The parent material fixed more of these components than did the detoxified derivative.

These results imply that, in addition to the dramatic loss of C'3t-fixing capacity during the detoxification procedures, there is also a loss or alteration of a substance or substances whose interaction with immune serum leads to fixation of C'1 and C'4.

Effect of serum dilution on complement fixation by endotoxins and endotoxoids. In the preceding experiments, the C'-fixing abilities of unmodified LPS and the several endotoxoids were compared in a reaction system arbitrarily designed to contain 0.1 ml of guinea pig serum (usually about 15 C' H_{50}) in a total volume of 1.0 ml. We wondered

TABLE 3. *Effect of rabbit anti-Serratia marcescens serum upon fixation of guinea pig complement by S. marcescens endotoxin and endotoxoid at 0 C (C' fixation expressed in 50% units)*[a]

Material tested	Antiserum dilution	C'	C'1	C'4	C'2	C'3t
Parent endotoxin	0[b]	<1.0	<1,000	1,125	<100	<8
	1:200	4.9	1,000	3,500	—	—
	1:100	7.5	1,500	4,100	<100	<8
	1:50	—	2,000	>4,200	—	—
	1:10	10.0	4,900	>4,200	<100	<8
Endotoxoid-2-A	0[b]	<1.0	<1,000	<500	<100	<8
	1:200	<1.0	<1,000	1,500	—	—
	1:100	3.2	<1,000	2,050	<100	<8
	1:50	—	<1,000	4,150	—	—
	1:10	12.4	>5,700	>4,200	<100	<8
Activity available	—	14.0	6,300	4,500	2,650	91.5

[a] *S. marcescens* endotoxin or endotoxid (10 μg) was incubated with approximately 14 C'H_{50} guinea pig C' in 1.0 ml of buffered saline at 0 C for 1 hr. Varying amounts of rabbit anti-*S. marscecens* serum were added. Residual C' and C' component hemolytic activities were measured by assay with intermediates.

[b] No antiserum added.

if the relative C′-fixing potency of these preparations would be different if they were assayed in different concentrations of guinea pig serum. Therefore, the C′-fixing abilities of a given amount (25 μg) of parent LPS, of endotoxoid-2-B, and of AHGG (for comparison) were studied in successively higher serum dilutions.

It was found that, in contrast to the endotoxoid and AHGG, LPS regularly fixed greater amounts of C′ when greater amounts of C′ were available (Fig. 2). LPS fixed more C′ than did the other preparations when the assays were performed in relatively undiluted serum, whereas AHGG actually fixed more C′ when very high serum dilutions were provided. The reason for the sharpened contrast in C′-fixing ability in undiluted serum is not yet clear. It may reflect the differential fixation of C′components known to be operative in the interaction of LPS and AHGG with the C′ system (1), or it may reflect a differential susceptibility of these preparations to the known C′-inhibiting effects of normal vertebrate serum (17, 18). In any case, the potent ability of unmodified LPS to fix C′ in amounts greater than endotoxoid and AHGG, was most dramatic when incubations were performed in relatively undiluted serum.

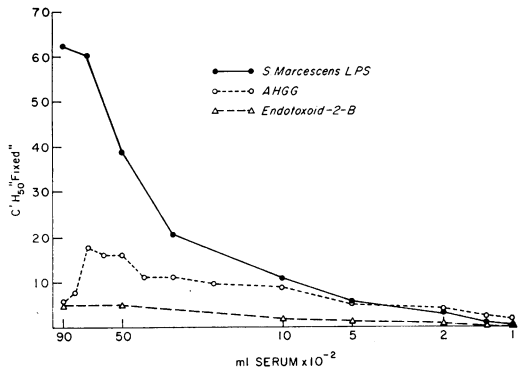

FIG. 2. *C′-fixing ability of 25 μg each of Serratia marcescens endotoxin, S. marcescens endotoxoid-2-B, and aggregated human γ-globulins (AHGG), in varying dilutions of guinea pig serum containing 16 C′H₅₀: 0.1 ml. All incubations were performed in a total volume of 1.0 ml.*

Complement fixation in undiluted serum from various mammalian species. In the final set of experiments, the ability of the *S. marcescens* endotoxin and endotoxoids to fix C′ in undiluted serum from various mammalian species was determined. This capacity was compared with the ability of certain other endotoxins, zymosan, sheep erythrocyte stroma, and AHGG to initiate and support such reactions with the C′ system in identical samples of undiluted serum.

The *S. marcescens* endotoxin was again active in fixing C′ in undiluted as well as diluted guinea pig serum (Table 4). At each dosage of LPS

TABLE 4. *Effect of endotoxins and endotoxoids upon hemolytic complement activity in undiluted mammalian sera (C′ fixation expressed in 50% units)*[a]

Material tested	Amt of material reacted with various sera						
	Guinea pig				Rabbit, 50 μg	Mouse, 50 μg	Human, 50 μg
	10 μg	50 μg	100 μg	500 μg			
Serratia marcescens endotoxin	73	168	>170	>170	>17.0	6.3	24.7
S. marcescens endotoxoids:							
Endotoxoid-1	<10	21	31	—	<2.0	2.4	8.9
Endotoxoid-2-A	12	23	23	—	<2.0	2.3	7.3
Endotoxoid-2-B	13	23	23	—	<2.0	<1.0	5.5
Endotoxoid-3	33	88	109	—	6.3	2.4	5.0
Endotoxoid-4	15	28	33	57	<2.0	2.2	7.0
Veillonella alcalescens endotoxin	68	118	>170	>170	10.0	>6.5	>34.6
Salmonella typhosa endotoxin	72	146	>170	>170	4.2	5.7	16.9
Zymosan	<10	<10	36	37	<2.0	2.1	9.7
Aggregated human γ-globulin	<10	21	32	62	5.0	4.3	7.7
Sheep erythrocyte stroma	<10	<10	<10	<10	<2.0	—	<4.0

[a] Endotoxins, endotoxoids, zymosan, aggregated human gamma globulins, and sheep erythrocyte stroma (0.1 ml) were incubated in 1.0 ml of guinea pig, rabbit, and human sera at 37 C for 1 hr. The C′ activity removed from the serum was determined. Incubations in mouse sera were performed in 0.5 ml for 30 min at 37 C. The activity available (C′ H_{50} per ml) was 200, 21.0, 7.5, and 41.7 for guinea pig, rabbit, mouse, and human sera, respectively.

tested, the parent material fixed more C′ than did the detoxified derivatives. Endotoxic LPS from *V. alcalescens* and *S. typhosa* also were very active in fixing C′ in undiluted guinea pig serum. By comparison, zymosan, sheep erythrocyte stroma, and AHGG, like the endotoxoids, were much less potent on a weight basis. Comparable results were obtained when the various preparations were tested against the serum of the rabbit or mouse (the species used most for determination of host reactivities to endotoxins), or of man (who, like the guinea pig, has a relatively well-defined C′ system). In each case, the unmodified LPS initiated much greater C′ fixation than did the endotoxioids, zymosan, sheep erythrocyte stroma, or heat-aggregated γ-globulins.

In summary, the ability to fix large amounts of hemolytic C′ in undiluted mammalian serum seems to be more characteristic of endotoxic LPS than of the other agents tested.

DISCUSSION

Several investigators have described chemical manipulations which result in selective loss of certain biological activities of endotoxic LPS (8, 15, 27–32, 46, 47). These studies were often directed toward vaccine development or chemical definition or both, of the toxic, haptenic, and tissue-binding portions of LPS. Decreased lethal, pyrogenic, and dermal toxicities were frequently observed after procedures which had only minimal effects upon antigenic, antibody-binding, tissue-binding, and nonspecific resistance-promoting properties. Such products have been termed endotoxoids (29).

Another characteristic of endotoxic LPS is its ability to induce fixation of C′ during incubation with serum of several mammalian species at 37 C (1, 11, 16, 24, 35, 36). In the experiments reported here, LPS induced fixation of C′ in amounts which greatly exceeded that fixed by zymosan, sheep erythrocyte stroma, or AHGG (Table 4). This effect was most apparent when assays were performed in undiluted serum (Fig. 2). The present investigation sought chiefly to discover whether this reactivity with C′ was altered by procedures which were known to result in detoxification of LPS.

In experiments performed at 37 C, endotoxoids produced by several distinct procedures (transesterification, deacylation, treatment with alkali, and treatment with pyridinium formate) were all deficient in their ability to fix C′ during incubations in normal mammalian serum. The capacity of unmodified *S. marcescens* LPS to fix C′ in normal serum, like that of unmodified *V. alcalescens* LPS (1), was almost entirely attributable to fixation of C′3t (Table 2). Activation of C′1, C′4, and C′2 may have been prerequisite to such reactivity with the C′3t complex, but the amounts fixed of these earlier-acting components were virtually undetectable in the assay systems used. Therefore, it was the capacity for fixation of C′3t that was most overtly decreased during the detoxification procedures. All of the C′3 components are probably involved in this LPS-C′ interaction, because, in addition to the pronounced fixation of C′3t, lesions indicative of terminal C′ component activity (Hadding, Müller-Eberhard, and Dalmasso, Federation Proc.**25**:485, 1966) are formed on the LPS surface (1; Humphrey, Dourmashkin, and Payne, *in press*). The facility with which unmodified LPS interacts with C′3t may have real biological significance, because such interactions are known to result in generation of a number of biologically active by-products capable of inducing host responses (6, 14, 25, 33, 39, 48, 49) which are prominent among the known biological effects of endotoxins (41, 52).

Endotoxoids could also fix large amounts of C′3t in experiments performed at 37 C, but *only* when immune serum was added to the preincubation mixtures. Upon addition of antiserum, however, large amounts of the early-acting C′ components also were fixed; thus, the ability to fix large amounts of C′3t, while fixing only minimal, if any, C′1, C′4, and C′2, remained peculiar to the biologically active LPS. Perhaps different sites on the LPS are utilized in the reaction between LPS and normal serum on the one hand and immune serum on the other.

In experiments performed at 0 C, under conditions where only C′1 and C′4 are fixed (19, 33; Table 3), the parent material could also fix more of these components in interaction with antibody and C′ than could the detoxified derivatives. This suggests that the modification of the LPS affects substrates which interact with C′ proximal to fixation of the C′3t components.

It seems likely that loss or alteration of substrates which support the binding or interactions, or both, of the C′ system (perhaps more than one step) occurs during the detoxification procedure. It had seemed earlier that antigenic structure might not greatly influence C′ fixation, and that the role of antigens in the C′ reaction was primarily the modification or aggregation of antibody molecules (12). Now it is known that at least three complement components, C′4, C′3 (B1C or C′3c), and C′9 (21–23), may interact directly with the erythrocyte surface. Perhaps additional C′ components also react in this manner. Like the red blood cell, endotoxic LPS presents a membranous substrate to the test serum. It is derived from the outer membrane of the bacterial cell, and electron microscopic studies have

revealed that it has a membranous structure (1, 2, 5, 20, 37, 38). The presence of a substrate which could either *initiate* the C′ sequence or favor C′ component interactions in the *initiated* C′ sequence, may contribute to the biological activities of LPS. Certainly, endotoxic LPS will be valuable in studies directed toward the definition of the chemical nature of the C′ substrates.

Again, it seems most likely that the decreased activity between the endotoxoids and C′3t is the end result of changes which mainly affect the earlier part of the LPS-(antibody)-C′ interaction. Haptenic material might, itself, not be modified but still result in decreased reactivity with C′ when it is not in its usual membranous milieu (T. Ishizaka, T. Tada, and K. Ishizaka, Federation Proc. **26**:530, 1967). Alternatively, haptens which react with immunoglobulins which are particularly efficient in their interactions with C′ (3, 13, 44) may have been lost selectively. It is also possible that substrates which are reported to activate C′1 either entirely independently of antibody (40) or which can modify the FC fragment (C′-binding site) independent of the classical antigen-combining site (4, 7) are lost during detoxification. The critical alterations even may involve substrates capable of activating C′3 independently of the earlier-acting C′ components, perhaps via the pathway used by the cobra venom C′3 activator (43).

The most significant change in the chemical structure of the endotoxic LPS during chemical detoxification is the cleavage of ester-bound long chain carboxylic acids. It has been repeatedly claimed that the presence of these ester-linked groups is essential for a "toxic conformation" (30, 31, 47). It is not yet clear whether these carbohydrate-fatty acid-ester functional groups are also receptor sites in the initiation or support or both, of fixation of C′ components.

Whatever the bases, it is clear that the C′-fixing ability of LPS is lost concomitantly with loss of certain of its biological activities. Therefore, it seems that the ability to fix C′ during preincubation with normal mammalian serum at 37 C may serve as an in vitro indicator of the detoxification of an LPS preparation. Further, the hypothesis that certain host reactivities to endotoxins may be mediated or potentiated by the C′ system (1, 11, 35, 42, 43, 50) is supported by these data.

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