INTERACTIONS OF THE COMPLEMENT SYSTEM WITH ENDOTOXIC LIPOPOLYSACCHARIDE: CONSUMPTION OF EACH OF THE SIX TERMINAL COMPLEMENT COMPONENTS*, ‡

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When endotoxic lipopolysaccharides (LPS) are incubated with fresh guinea pig serum, a strong interaction between LPS and the complement (C′) system ensues. C′ is "fixed" or consumed (2, 3) while lesions which relate to the activity of the terminal C′ components (4, 5) appear on LPS (2, 6.) In this interaction pronounced consumption of the classical terminal C′ component (C′3) is observed, despite only minimal consumption of the earlier-acting C′1, C′4, and C′2) (2, 3, 7, 8). The classical C′3 activity (hereafter referred to as "C′3–C′9") is now known to consist of at least six discrete proteins, termed C′3, C′5, C′6, C′7, C′8, and C′9 (9–11). These components seem to subserve most of the biological functions presently attributed to C′ (9, 10).

These considerations have led us to investigate the interaction of LPS with C′3–C′9. We sought to determine whether LPS induced consumption of the entire C′3–C′9 sequence or merely initiated the consumption or inactivation of a single member protein. The consumption profile of the nine C′ components was determined during the interaction of both LPS and preformed immune complexes with fresh guinea pig serum. The results clearly show that despite its inability to induce substantial consumption of C′1, C′4, and C′2, LPS (like immune complexes) effectively induces consumption of *each* of the terminal six C′ components.

Materials and Methods

Endotoxic LPS.—LPS was isolated from *Veillonella alcalescens* strain V5 by the phenol-water extraction procedure of Westphal and Lüderitz (12).

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

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Aggregated Human Gamma Globulins (AHGG).—AHGG were prepared by heating of the Cohn fraction II of human serum (Hyland Laboratories, Los Angeles, Calif.) for 20 min at 63°C.

Immune Complexes of Bovine Serum Albumin (BSA) and Rabbit Antiserum (Rabbit Anti-BSA).—Crystalline BSA was obtained from Pentex Incorporated, Kankakee, Illinois. Rabbit anti-BSA was obtained from Hyland Laboratories, Los Angeles, Calif., and contained 240 μg antibody nitrogen per ml.[1] BSA and rabbit anti-BSA were reacted at equivalence for 24 hr at 4°C in the presence of 0.01 M EDTA. The precipitate was washed twice in EDTA-saline

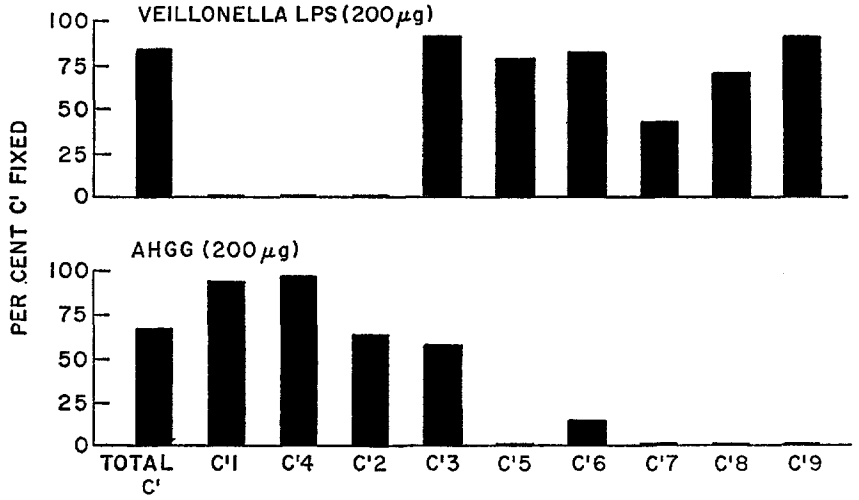

FIG. 1. "Fixation" (consumption) profiles of total C′ and the nine known C′ components in guinea pig serum (0.1 ml in a total volume of 1.0 ml) upon interaction with 200 μg *Veillonella alcalescens* lipopolysaccharide (LPS) and 200 μg aggregated human gamma globulins (AHGG) during 1 hr incubations at 37°C. Similar results were obtained when smaller amounts of LPS and AHGG (10, 25, and 100 μg, respectively) were tested in this manner.

and resuspended in saline. Final protein concentrations were determined by the assay of Lowry *et al.*, (13).

Guinea Pig Serum.—Pooled guinea pig serum was obtained from Texas Biologicals, Inc., Fort Worth, Texas, and maintained at −70°C.

Complement Consumption ("Fixation").—The ability of LPS, zymosan, AHGG, and BSA–anti-BSA complexes to fix C′ was tested in diluted (1:10) and/or undiluted guinea pig serum. These test reagents (0.1 ml) were reacted with either 0.1 ml guinea pig serum and 0.8 ml veronal-buffered saline, or with 0.9 ml guinea pig serum for 1 hr at 37°C. Residual total C′ and C′1, C′4, C′2, and C′3–C′9 (C′-EDTA) activities were measured by minor modifications (14) of the methods of Mayer (15) and Nelson et al., (11). Residual C′3, C′5, C′6, C′7, C′8, and C′9 activities were quantitated by minor modifications[2] of the methods of Nelson et al. (11).

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[1] Kindly determined by L. Lichtenstein.

[2] Shin, H. S., and M. M. Mayer. Manuscript in preparation.

TABLE I

*Comparison of the Effects of V. alcalescens LPS, Zymosan and Preformed Complexes of BSA–Rabbit Anti–BSA upon C′ Component Hemolytic Activities in Undiluted Guinea Pig Serum (C′ "Fixation" Expressed as Per cent of Available C′ Fixed)**

Material tested	Amount	Total C′	C′1	C′4	C′2	C′3	C′5	C′6	C′7	C′8	C′9	C′-EDTA
	μg											
LPS	10	10	<10	<10	<10	45	30	36	27	19	24	19
LPS	25	25	<10	<10	<10	61	26	45	35	25	33	52
LPS	50	38	<10	<10	<10	69	30	45	39	23	32	65
LPS	100	43	<10	<10	<10	83	41	54	54	23	51	86
LPS	200	>67	<10	<10	<10	88	46	77	74	25	68	89
Zymosan	25	<10	<10	<10	<10	13	13	13	5	13	3	<10
Zymosan	100	<10	<10	<10	<10	22	12	25	19	15	4	22
Zymosan	200	21	<10	<10	<10	24	14	28	26	14	5	30
Zymosan	500	41	<10	<10	<10	49	36	36	33	15	6	58
BSA–anti-BSA	33	<10	<10	<10	<10	14	13	18	4	N.D.‡	9	19
BSA–anti-BSA	65	26	14	<10	<10	27	17	18	5	10	8	43
BSA–anti-BSA	130	43	51	17	20	57	24	27	10	12	N.D.‡	49
BSA–anti-BSA	260	63	77	40	39	68	28	28	15	27	21	69
None	—	0	0	0	0	0	0	0	0	0	0	0
Activity available§		126	56,700	40,500	23,850	10,000	40,000	10,000	80,000	300,000	130,000	819

* *V. alcalescens* lipopolysaccharide (LPS), zymosan, and preformed immune complexes of bovine serum albumin (BSA) and rabbit–anti-BSA were incubated with 0.9 ml guinea pig C′ in 1.0 ml total volume at 37°C for 1 hr. Residual C′ hemolytic activities were measured by assay with intermediates and the per cent of the available C′ fixed was calculated.

‡ N. D., not done.

§ Total C′, C′1, C′4, C′2, and C′-EDTA expressed in 50% hemolysis units. C′3, C′5, C′6, C′7, C′8, and C′9 expressed in 63% hemolysis units (15).

RESULTS

The effect of LPS upon each of the six terminal C′ components first was tested in 37°C reaction mixtures (1.0 ml) which contained 0.1 ml guinea pig serum. Under these conditions, LPS induced substantial consumption of each C′3, C′5, C′6, C′7, C′8, and C′9, with only limited consumption of C′1, C′4,

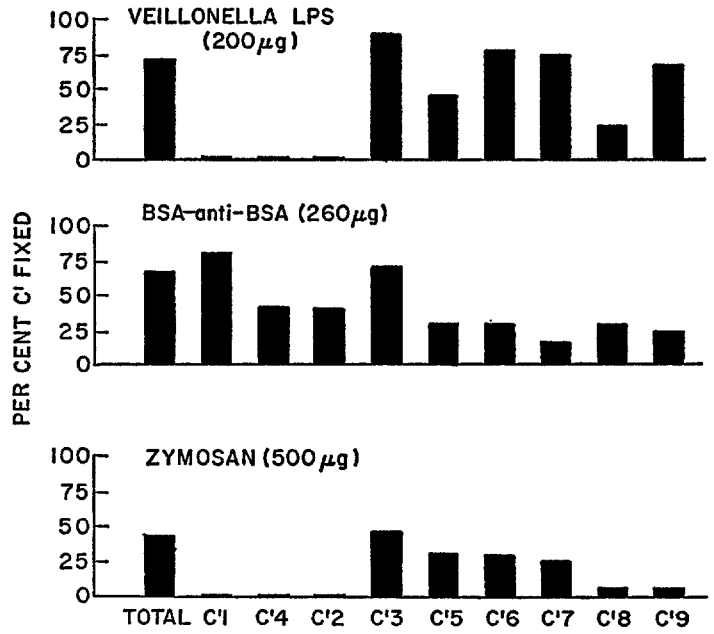

FIG. 2. "Fixation" (consumption) profiles of total C′ and the nine known C′ components in more concentrated guinea pig serum (0.9 ml in a total volume of 1.0 ml) upon interaction with 200 μg *Veillonella alcalescens* lipopolysaccharide (LPS), 260 μg washed preformed immune complexes of bovine serum albumin (BSA) and rabbit antiserum (anti-BSA) reacted at equivalence, and 500 μg zymosan during 1 hr incubations at 37°C. These profiles represent the highest dosages tested in the experiments shown in Table I.

and C′2 (Fig. 1). Hence, the entire C′3–C′9 pathway was utilized during this LPS-serum interaction even though there was minimal detectable consumption of the C′ components involved in the formation of the known C′3-converting enzyme ("C′4, 2a" or C′3 convertase") (16). Conversely, AHGG consumed large amounts of C′1, C′4, and C′2, but relatively little consumption of the individual C′3–C′9 components ensued. Indeed, substantial consumption only of the C′3 component was detected in the reaction mixtures; the titers of the later-acting C′3–C′9 components were virtually unchanged (Fig. 1).

It is known that the pattern of consumption of the classical C′ components may differ depending upon whether incubations are performed in diluted or

undiluted serum (3, 17, 18). Therefore, the interactions of the C′ system with graded amounts of LPS, zymosan and immune complexes were tested in undiluted guinea pig serum. Again, despite minimal consumption of C′1, C′4, and C′2, even small amounts of LPS had potent capacity to bring about fixation of *each* of the six terminal C′ components (Table I; Fig. 2). Zymosan also brought about consumption of each of the C′3–C′9 components with only minimal detectable uptake of the earlier-acting C′ components; however, on a weight basis much larger amounts of zymosan than LPS were required to consume equal amounts of the terminal C′ components. Immune precipitates also induced substantial consumption of the C′3–C′9 components, but in the process large amounts of C′1, C′4, and C′2 were consumed.

It should be noted that incubation of LPS with purified preparations of any of the six terminal C′ components did not lead to their consumption; hence, other serum factors were needed. Whether these included the earlier-acting C′ components is not yet clear.

DISCUSSION

Previous investigations have shown that endotoxic LPS fixes large amounts of classical C′3 during incubation with guinea pig serum, despite minimal consumption of C′1, C′4, and C′2 (2, 3). Classical C′3 now is known to consist of at least six separate proteins (9–11). The appearance of characteristic C′-mediated lesions on LPS during its reaction with guinea pig serum (2, 6, 19)—an event which on the erythrocyte is associated with the activity of the terminal C′ components (4, 5)—indirectly suggested the C′ sequence reached completion on LPS. The chief purpose of the present investigation was to determine directly whether substantial consumption of each of the six terminal C′ components occurs during the LPS–guinea pig serum interaction.

It was found that each of the six C′3–C′9 components was consumed even by small amounts of LPS (10–25 μg) during these incubations. Given amounts of LPS consumed greater quantities of the C′3–C′9 components than did immune complexes or AHGG, even though they consumed much smaller quantities of the earlier-acting C′ components. Such a relatively "preferential" consumption of classical C′3 during serum-polysaccharide (20) and serum-lipopolysaccharide interactions (2, 3, 7, 8) had previously been observed, as had relatively preferential consumption of the earlier-acting C′ components during incubations of serum with preformed immune complexes and AHGG (2, 3, 8, 20–23).

Earlier it had been found that the addition of hyperimmune rabbit anti-endotoxin serum to incubation mixtures of normal guinea pig serum and LPS led to consumption of large amounts of C′1, C′4, and C′2, as well as to consumption of classical C′3 (C′3–C′9) (3). As expected, LPS reacting with *hyperimmune* serum leads to a C′ consumption profile similar to that induced by the im-

mune complexes. Whether this effect of heterologous hyperimmune serum relates to the nature or the amounts of the immunoglobulins present, to the antigenic sites against which they are directed, or to the species of origin is not yet clear.

When highly purified preparations of any of the six terminal C′ components were incubated with LPS, no consumption occurred which implied that other serum factors were needed. The nature of these other factors in the LPS-normal serum interaction is not yet clear and is beyond the scope of the present investigation. Even though LPS can efficiently consume the C′3–C′9 components without apparent consumption of C′1, C′4, and C′2, these early-acting factors cannot be excluded as a pathway to consumption of C′3–C′9 since LPS might promote extremely efficient convertase (C′4, 2a) formation. On the other hand, there could be other pathways to consumption of the C′3–C′9 components.

These investigations suggest LPS may be a valuable reagent in the study of the interactions of the C′3–C′9 components and in the chemical definition of the cellular C′ substrates (2, 3, 6, 24), the activated C′3–C′9 components and the biologically active "split products" derived from them (2, 25, 43). Since LPS is derived from the outer bacterial membrane (26–28) and itself has membrane-like structure (2, 28–30), perhaps it will serve as a model of C′-membrane interactions in general.

The ability of LPS to induce fixation of the six terminal C′ components may have real biological meaning, because these components are known to subserve several activities including immune adherence agglutination of platelets (7, 31) and neutrophils (32), generation of vasoactive factors with anaphylatoxic (17, 33–35) and neutrophil chemotactic (36, 37) properties, and cytocidal reactions (9–11, 15). Since these modalities are prominent among the events which follow the injection of LPS into several experimental animals (38, 39), it may be through the C′ system that certain of the biological effects induced by LPS are mediated (2, 3, 40–42). Indeed, ongoing studies in these laboratories have shown that the interaction of LPS with fresh guinea pig serum leads to generation of anaphylatoxin(s) and neutrophil chemotactic factor(s) via the C′ system (25, 43). It is possible that the property of consuming the C′3–C′9 components, common to LPS and immune complexes, accounts for some of the known similarity (44, 45) in reactivities which they evoke in various animal species.

SUMMARY

Large amounts of *each* C′3, C′5, C′6, C′7, C′8, and C′9 were consumed when guinea pig serum was incubated with endotoxic lipopolysaccharide, zymosan, or preformed immune complexes. Since these C′ components subserve several of the biological activities which follow the injection of endotoxins into experi-

mental animals, these experiments support the hypothesis that certain biological effects induced by endotoxins may be mediated via the C′ system, and may account for some of the known similarity in the reactivities evoked by endotoxins and immune complexes in vivo.

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