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ENHANCEMENT OF PLATELET RESPONSE TO IMMUNE COMPLEXES AND IgG AGGREGATES BY LIPID A-RICH BACTERIAL LIPOPOLYSACCHARIDES*‡

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IgG aggregates and immune complexes have been shown to stimulate cells in vitro (1–3). This includes the platelet, a cell that secretes a variety of constituents in response to these stimuli. For example, human platelet secretion is initiated by immune complexes and IgG aggregates (1), by aggregated Fc fragments (4, 5) but not aggregated $F(ab')_2$ fragments, leading to the concept of a platelet "Fc receptor" (5, 6). This concept has recently been strengthened by the demonstration that aggregated IgG binds to human platelets and that binding is inhibited more strongly by human IgG than by $F(ab')_2$ fragments or bovine IgG (7). The nature of the postulated platelet Fc receptor remains unclear, but the fact that the first component of the classical complement pathway, C_1, like the platelet, is activated by aggregated IgG or Fc fragments, has led to the hypothesis that C_1 bound to the platelet surface plays a critical role in the platelet response to aggregated IgG (8). There is, however, little information on the effects of other activators of C_1 on platelet function.

The lipopolysaccharide (LPS)[1] component of gram-negative bacteria is a molecule that may be responsible for many of the pathogenic properties of the whole organism (9). LPS from smooth bacterial strains consists of a type-specific O antigen, a strain-specific core polysaccharide, a trisaccharide of 2-keto-3-deoxy octulosonate (KDO), and a lipid termed "lipid A" (10). The KDO-lipid A portion of the molecule is remarkably similar in most bacterial strains (9) and may be obtained in isolated form as the LPS of heptose-deficient rough mutants such as *Salmonella minnesota* R595. This lipid-rich LPS and lipid A isolated by acid hydrolysis of LPS (10) have been shown to provide many of the strain independent activities of LPS (11). Both lipid A and lipid A-rich LPS from *S. minnesota* R595 have recently been shown to activate complement via C_1 (12,

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; KDO, 2-keto-3-deoxy octulosonate; LDH, lactic dehydrogenase; LPS, lipopolysaccharide; TBS, 30 mM Tris, 120 mM NaCl, 5 mM glucose.

13). In this paper, we report data showing that lipid A and lipid A-rich LPS from *S. minnesota* R595 (LPS R595) by themselves are not potent platelet stimuli. These bacterial products are shown, however, to dramatically potentiate the effectiveness of IgG aggregates and immune complexes as initiators of the platelet release reaction.

Materials and Methods

Chemicals. Phenol and chloroform were from Mallinckrodt Inc. (St. Louis, Mo.) and the petroleum ether (bp = 20–40°C) from J. T. Baker Chemical Co. (Phillipsburg, N. J.). Schwartz Mann Div. Becton, Dickinson & Co. (Orangeburg, N. Y.) provided the tris hydroxymethyl amino methane (Tris, Ultra Pure) and E. R. Squibb & Sons (Princeton, N. J.) provided the human Cohn fraction II (lot no. 2030). The [^{3}H]serotonin binoxalate (1 Ci/mM) in 2% ethanol and the ^{131}I (12–25 Ci/mg) were from New England Nuclear (Boston, Mass.). Bovine serum albumin (BSA; was recrystallized three times and was from Armour Pharmaceuticals Company (Phoenix, Ariz.).

Lipopolysaccharide. LPS of *S. minnesota* R595 was extracted from bacterial paste (New England Enzyme Center, Boston, Mass.) by the phenol-chloroform-petroleum ether procedure (14). The purified LPS was suspended at a concentration of 2.5 mg/ml in 0.9% NaCl containing 0.1% triethylamine, sonicated, and subsequently dialyzed extensively against 0.9% NaCl to remove unbound triethylamine. Lipid A was prepared by acid hydrolysis (15) of *Salmonella typhimurium* R60 LPS and dispersed in 1% triethylamine and dialyzed against 0.9% NaCl. 0.1–1% triethylamine alone had no effect on platelet serotonin release in the presence or absence of IgG aggregates.

Gamma Globulin. Purified polyclonal gamma globulin was obtained by diethylamino ethyl cellulose chromatography of Cohn fraction II. This material produced a single precipitin arc on immunoelectrophoresis at 20 mg/ml when reacted with goat whole anti-human serum. IgG myeloma proteins of greater than 95% homogeneity by sodium dodecyl sulfate polyacrylamide gel electrophoresis were generously provided by Dr. Hans Spiegelberg of the Scripps Clinic. Proteins were heat aggregated at 63°C for 30 min at 20 mg/ml in 0.01 M sodium phosphate 0.15 M NaCl pH 7. Isolated soluble IgG aggregates were prepared by fractionation of heat-aggregated IgG on a 4.5 × 75-cm column containing Ultrogel Ac A22 (LKB Instruments, Inc., Rockville, Md.). Protein eluting at apparent mol wt of 500,000–1,500,000 daltons was pooled, frozen, and stored at −70°C until use.

Other Platelet Stimuli. Monosodium urate crystals were prepared as described (16). Insoluble human skin collagen extracted by the Nishihara procedure was generously provided by Dr. George Wilner through Dr. John Griffin of the Scripps Clinic. Crude bovine thrombin was from Parke, Davis & Company (Detroit, Mich.). Anti-human platelet antibodies were raised in New Zealand white rabbits (Rancho de Conejo, La Jolla, Calif.). These antisera were absorbed with human red blood cells until they did not agglutinate human neutrophils or red blood cells. In addition, no precipitin lines were observed when these antisera were reacted against human plasma in immunodiffusion analysis.

Protein Labeling. IgG was labeled with ^{131}I by the procedure of McConahey and Dixon (17).

BSA-Anti-BSA Complexes. Antisera to BSA were raised in New Zealand white rabbits (Rancho de Conejo) and contained 417 μg of antibody nitrogen/ml by quantitative precipitin analysis. Immune complexes were precipitated at equivalence using this antiserum and washed twice in 0.15 M NaCl 0.01 M sodium phosphate pH 7 before platelet release experiments.

Platelet Release Experiments. These were done with plastic apparatus. Blood from normal donors who had taken no medication in the previous week was drawn through a 20-gauge needle into 1/6 vol acid citrate dextrose (18). Platelet-rich plasma was isolated by centrifugation at 200 *g* in an International centrifuge (International Equipment Co., Boston, Mass.) for 15 min at room temperature and was incubated at 37°C for 30 min with 0.1 μCi/ml [^{3}H]serotonin resulting in uptake of 80–90% of radioactivity. Platelets were pelleted by centrifugation at 1,000 *g* for 15 min at 10°C in an International centrifuge, and the platelet button resuspended in 30 mM Tris, 120 mM NaCl, 5 mM glucose (TBS) pH 6.5. The platelets were then recentrifuged and resuspended twice before being centrifuged and finally suspended at 2 × 10^8 cells/ml in TBS pH 7.4. These suspensions contained less than one white cell per 2,000 platelets.

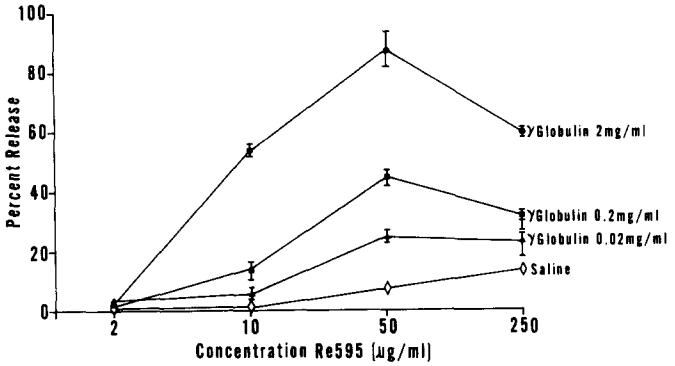

FIG. 1. Response of platelets to LPS R595. LPS R595 at the final concentration shown on the abscissa was mixed with varying quantities of polyclonal human IgG. Platelet suspensions were added and percent serotonin release determined after a 15-min incubation at 37°C. (Preliminary studies had shown a plateau in serotonin release induced by these stimuli after a 10-min incubation). Mean ± SEM of triplicate determinations. Where no error bars appear, the error is within the point.

In general, 100 µl of test substance was added to 12 × 75-mm Falcon polystyrene (Sherwood Medical Industries, Inc., St. Louis, Mo.) tubes and incubated at 37°C for 10 min, at which time 400 µl of the prewarmed platelet suspension was added and the mixture incubated with shaking at 37°C in a Dubnoff metabolic incubator. At appropriate times, the tubes were placed into ice for 2–5 min and centrifuged at 5,000 *g* for 15 min at 4°C. To 2 ml of a mixture of equal parts Aquasol (New England Nuclear) and toluene, 100 µl of supernate was added and the mixture counted in a Searle Delta 300 liquid scintillation spectrometer (Searle Analytic Inc., Des Plaines, Ill.). Percent-specific release of serotonin was defined as:

$$\frac{\text{radioactivity in stimulus-treated supernate} - \text{radioactivity in buffer-treated supernate}}{\text{total radioactivity releasable by 0.2\% Triton X-100} - \text{radioactivity in buffer-treated supernate}} \times 100$$

In some experiments, loss of lactic dehydrogenase (LDH) was assayed as well (16). Data were analyzed only from those platelet preparations in which less than 10% of total serotonin or LDH was in buffer-treated supernates.

Results

Effect of LPS R595 and Lipid A on Washed Human Platelets. The ability of LPS R595 and lipid A to initiate serotonin secretion from washed human platelets was studied. Fig. 1 shows that addition of mixtures of human gamma globulin preparations (which induced little serotonin release by themselves) and LPS R595 to the cells resulted in significant serotonin release at LPS concentrations as low as 10 µg/ml. The cytoplasmic enzyme, LDH, was retained at all doses of LPS and gamma globulin employed. The LPS alone showed little activity at the concentrations tested. Similar data were obtained with platelets washed by the techniques of Mustard et al. (19) and Walsh (20). The lipid A preparation was comparable to LPS R595 in activity (not shown).

The gamma globulin preparations may have contained antilipid A antibodies (21) which in the presence of lipid A could have formed immune complexes, thus leading to the observed platelet stimulation. To explore this possibility, the nature of the active material in the gamma globulin preparation was studied.

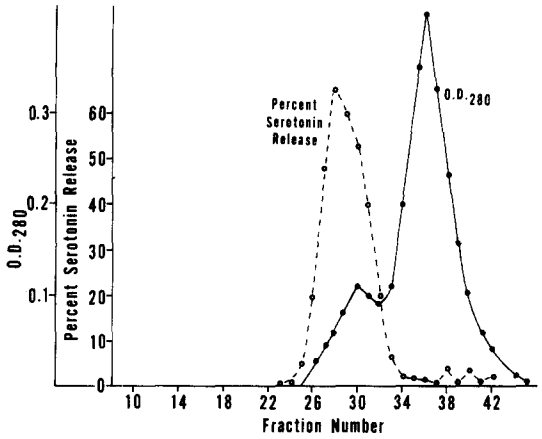

FIG. 2. Size of the component of gamma globulin in preparation inducing serotonin release in the presence of LPS R595. 10 ml of 20 mg/ml gamma globulin were placed on a 4.5 × 100-cm column containing Sephadex G200, and 20-ml fractions collected. 100 μl of each fraction was mixed with 50 μl 500 μg LPS R595/ml. Percent serotonin release was determined 15 min after addition of 350 μl of platelet suspension. Each point is the mean of duplicate determinations (O-----O, percent serotonin release; ●——●, absorbance at 280 nm).

Size of the Active Material in Gamma Globulin Preparations. When the gamma globulin preparations were passed over a Sephadex G200 column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; Fig. 2), serotonin releasing activity in the presence of LPS R595 appeared only in fractions eluting before IgG monomer. This suggested that the active material was IgG aggregates or a large non-IgG trace contaminant. To help distinguish these possibilities, the inactive fraction containing IgG monomer was heated at 63°C. Heating for as little as 5 min resulted in serotonin releasing activity when the protein was mixed with LPS R595. This suggested that LPS R595 worked to enhance the platelet's response to IgG aggregates. The remote possibility that heating activated some previously inert contaminant, or altered antilipid A antibodies, still remained. To rule out this possibility, the effect of LPS R595 platelet response to heated myeloma proteins was assessed.

IgG myeloma proteins were heat aggregated at 63°C for 30 min. Fig. 3 shows that LPS R595 enhanced platelet serotonin release in response to these heat-aggregated proteins. This provided strong evidence that LPS R595 or lipid A effect on platelets was mediated through IgG.

Effect of LPS R595 and Lipid A on Platelet Response to IgG Aggregates. Because our polyclonal gamma globulin preparations and heated myeloma proteins were mixtures of IgG monomer and aggregates, the effects of LPS R595 and lipid A on platelet response to purified IgG aggregates were quantitated. These results are shown in Fig. 4. LPS R595 or lipid A induced a 50-fold increase in platelet sensitivity to IgG aggregates.

Effect of Size of IgG Aggregates on Platelet Stimulation in the Presence and Absence of LPS. Because IgG aggregates bind to LPS R595 and lipid A,[2] the

[2] M. H. Ginsberg and D. C. Morrison. The binding of aggregated IgG to lipid A-rich bacterial lipopolysaccharide. Submitted for publication.

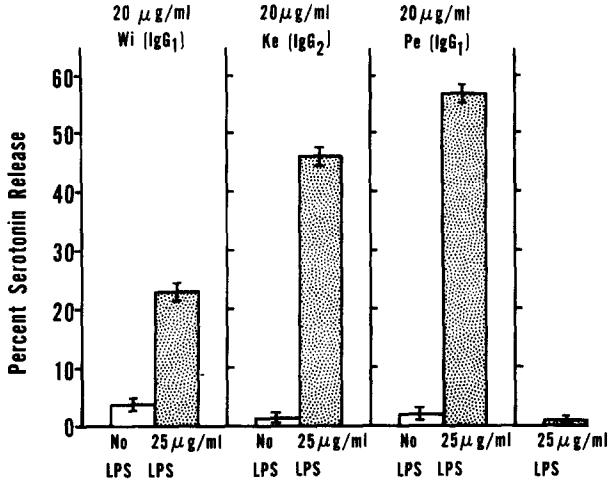

FIG. 3. Effect of LPS R595 on platelet response to heat-aggregated myeloma proteins. 50 μl of 250 μg/ml LPS R595 or of saline was mixed with 50 μl of 200 μg/ml heat-aggregated myeloma proteins. Percent serotonin release determined 15 min after addition of 400-μl platelet suspension. Mean ± SEM of triplicate determinations.

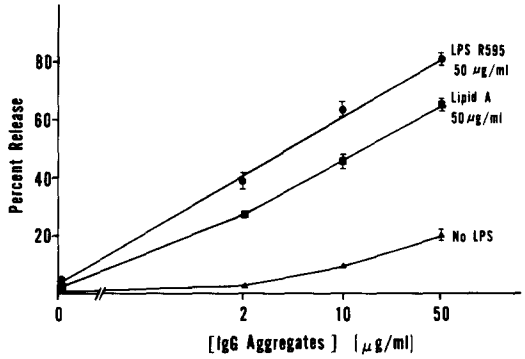

FIG. 4. Effect of LPS R595 and lipid A on platelet response to soluble polyclonal IgG aggregates. 50 μl of LPS R595 (○——○) or lipid A (■——■) at 500 μg/ml was mixed with 50 μl soluble IgG aggregates (final concentration shown on abscissa). Percent serotonin release was determined 15 min after addition of 400-μl platelet suspension. Mean ± SEM of triplicate determinations.

possibility arises that this enhancement is due to increased apparent size of aggregates in the presence of lipid A. To examine this, the size requirements for IgG aggregate-induced platelet stimulation in the presence and absence of LPS were studied. To assess these requirements, ^{131}I-IgG was heated at 63°C for 30 min and fractionated by gel filtration (Fig. 5) chromatography. Serotonin releasing activity was present in fractions larger than IgG monomer and was enhanced by the addition of LPS R595. Subsequently, dilutions of fractions corresponding in size to large (apparent M_r $>10^6$ daltons – fraction 26) soluble aggregates, small (apparent M_r 3–6 × 10^5 daltons – fraction 38) soluble aggregates, and IgG (fraction 46) monomer were added to platelet suspensions in the presence and absence of LPS. Inasmuch as IgG aggregates are unstable, the sizes of these three aggregate populations were reassessed on sucrose density gradients

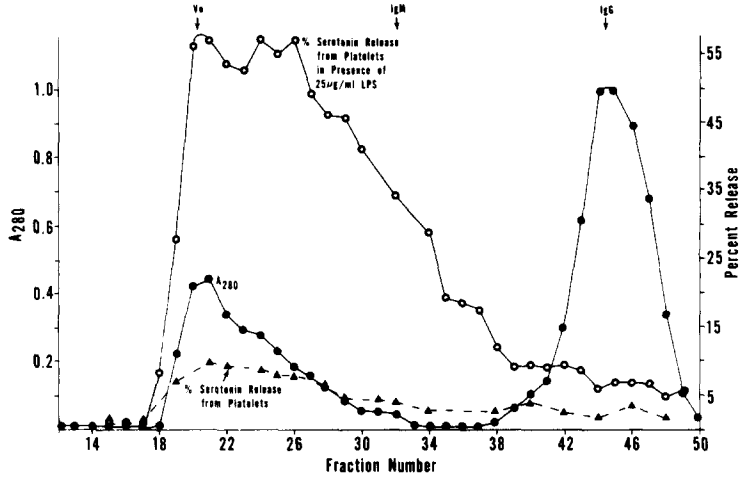

FIG. 5. Gel filtration of heated gamma globulin. ^{131}I-polyclonal gamma globulin was heated at 20 mg/ml 63°C for 30 min. 10 ml was then placed on a 4.5 × 80-cm column containing Ultrogel Ac A22 and 20-ml fractions collected. Protein was determined by absorbance at 280 nM (●——●) or radioactivity (not shown). 50 μl of each fraction was mixed with 50 μl 0.9% NaCl (△-----△) or 250 μg/ml LPS R595 in saline (○——○), and percent serotonin release determined 15 min after addition of 400-μl platelet suspension. Results are mean of duplicate determinations.

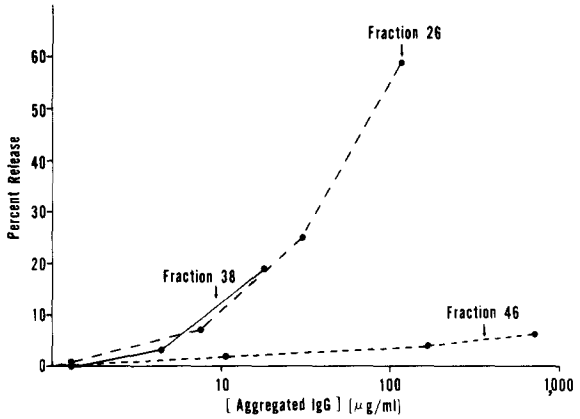

FIG. 6. Response of platelets to various sized IgG aggregates. 100 μl of various dilutions of IgG fractions (see Fig. 5) were added to 50 μl 0.9% NaCl, and percent serotonin release determined 15 min after addition of 350-μl platelet suspension. Protein concentrations represent the final concentration in the 500-μl incubation mixture. Results are mean of duplicate determinations (●····●, fraction 26; ●——●, fraction 38; ●-----●, fraction 46).

at the time they were added to the platelet suspensions. Fig. 6 shows that, in the absence of LPS, small soluble IgG aggregates were as effective on a weight basis as larger aggregates in stimulating platelets. Fig. 7 indicates that in the presence of LPS R595, the small and large aggregates were again approximately equally effective and some 20-fold more active than the same proteins in the absence of LPS. Fig. 8 documents the size of the various column fractions, as assessed on sucrose density gradients in the absence of LPS, at the same time they were added to the platelet suspensions. Because LPS R595 selectively

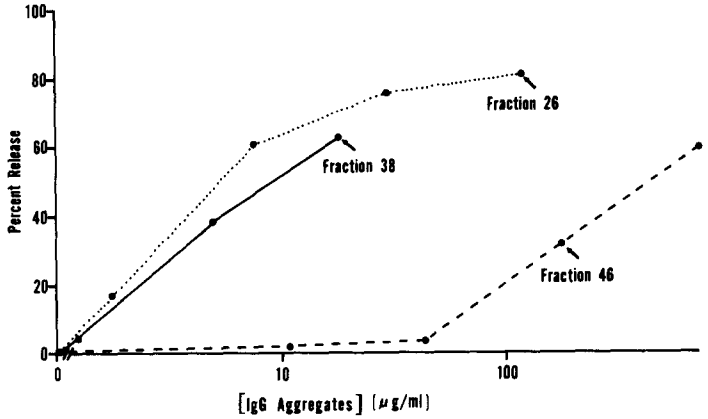

FIG. 7. Response of platelets to various sized IgG aggregates in the presence of LPS R595. 100 μl of various dilutions of IgG fractions (see Fig. 5) was added to 50 μl 250 μg/ml LPS R595 in saline, and percent serotonin release determined 15 min after addition of 350-μl platelet suspension. Protein concentrations represent the final concentration in the 500-μl mixture. Results are means of duplicate determinations (●· · · ·●, fraction 26; ●——●, fraction 38; ●-----●, fraction 46).

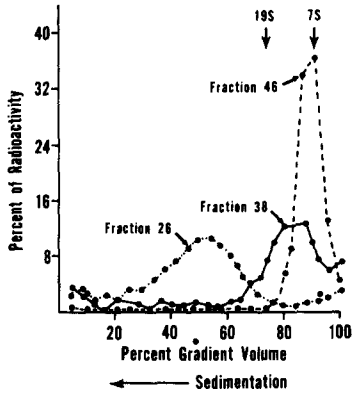

FIG. 8. Migration of various sized IgG fractions on sucrose density gradients. 0.4 ml of ^{131}I-IgG fractions from Fig. 5 was placed on 5–20% linear sucrose gradients at the same time the experiments in Figs. 6 and 7 were performed. They were then centrifuged at 20,000 rpm for 18 h in a SW 50.1 rotor at 4°C. Fractions were collected from the bottom of the tube. Greater than 80% of applied radioactivity from each gradient was recovered.

binds IgG aggregates (see fn. 2), these same fractions were analyzed on sucrose gradients in the presence of LPS R595. All of the radioactivity in fraction 26 was bound by the LPS whereas only 60% of that in fraction 38 and none of that in fraction 46 was bound. The unbound material in fraction 38 formed a homogeneous 7S peak. Thus, protein concentrations in fraction 38 were corrected for monomer contamination by multiplying by 0.6. These experiments indicate that small and large soluble IgG aggregates are equally effective on a weight basis in stimulating the platelet, and their effects are equally enhanced by LPS R595. These data suggest that simple increase in the apparent size of soluble IgG aggregates is not sufficient to explain enhanced cell stimulation.

Effect of LPS R595 on Response of Platelets to BSA-Anti-BSA Immune

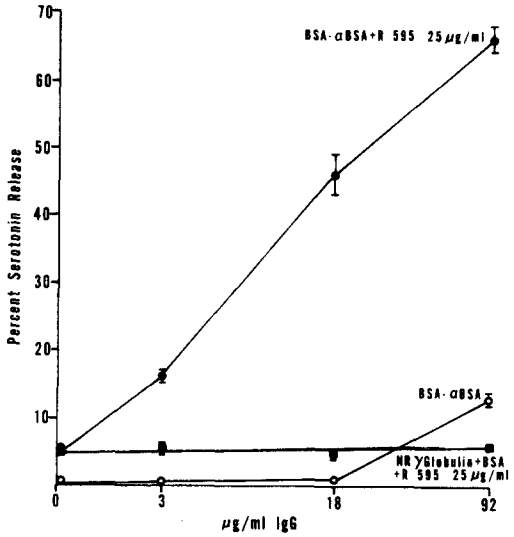

FIG. 9. Effect of LPS R595 on platelet response to BSA-anti BSA immune complexes. 50 μl of various dilutions of insoluble BSA-anti-BSA immune complexes was mixed with 50 μl 0.9% NaCl (○——○) or 250 μg/μl LPS R595 in 0.9% NaCl (●——●). In addition, 50 μl of dilutions of rabbit gamma globulin plus BSA in 0.9% NaCl was added to 50 μl 250 μg/ml LPS R595 (■——■). Percent serotonin release was determined 15 min after addition of 400-μl platelet suspension. Mean ± SEM of triplicate determinations.

Complexes. It is possible that LPS R595 acted by effectively insolubilizing soluble IgG aggregates. To rule this out, (since immune complexes have similar biologic properties to IgG aggregates and are initiators of tissue injury in vivo) the effect of LPS on platelet response to insoluble immune complexes was examined. Fig. 9 shows the effect of LPS R595 on human platelet secretion induced by insoluble rabbit BSA-anti-BSA complexes. The LPS R595 induced a 25-fold enhancement in platelet sensitivity to these immune complexes, but had little effect on a mixture of BSA and normal rabbit gamma globulin.

Effect of LPS R595 on Other Platelet Stimuli. LPS R595 and lipid A increased platelet sensitivity to aggregated IgG and immune complexes, leading us to examine their effects on other stimuli of platelet secretion.

Fig. 10 shows that LPS R595 slightly inhibited platelet serotonin release induced by a proteolytic enzyme, thrombin, a negatively charged particle, the monosodium urate crystal (16), or antiplatelet antibody. The response to insoluble human skin collagen was slightly enhanced. In contrast, as before, the response to IgG aggregates was markedly enhanced. This indicates that LPS R595 does not engender a state of "generalized cellular hypersensitivity" and that its effects on platelet stimulation are relatively specific for immune complexes.

Discussion

The data presented in this report show that the common lipid region of bacterial LPS markedly amplifies immune complex-induced platelet serotonin release. The release of radioactive serotonin as measured here, reflects the fate of the endogenous platelet amine (22). Synergistic release by LPS and aggre-

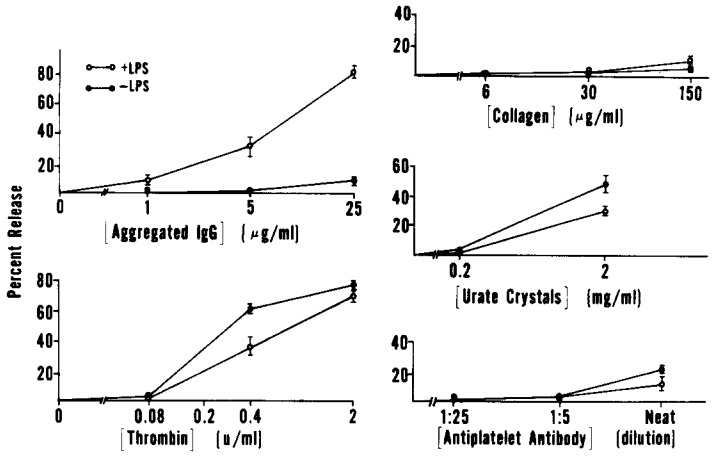

FIG. 10. Effect of LPS R595 on other platelet stimuli. 50 μl of a solution containing 500 μg/ml LPS R595 in 0.9% NaCl or 0.9% NaCl was added to 50 μl of various concentrations of aggregated IgG, thrombin, insoluble human skin collagen, antiplatelet antibody, or monosodium urate crystals in 0.9% NaCl. Percent serotonin release was determined 15 min after addition of 400-μl platelet suspension, mean ± SEM of triplicate determinations. Concentrations indicated are those achieved in the final 500-μl incubation mixture (●——●, stimulus alone; ○——○, stimulus + LPS R595).

gated IgG probably represents a true secretory reaction as other granule constituents are released[3] and cytoplasmic LDH is retained under these circumstances.

IgG monomer preparations were some 50-fold less active than aggregates in inducing platelet serotonin release in the presence of lipid A or LPS R595. This may be the result either of minimal serotonin releasing activity on the part of IgG monomer, or a 2% contamination (which would not have been detectable in our system) of the monomer preparation with soluble IgG aggregates. Heating monomer to 63°C markedly increased its activity in inducing platelet serotonin release, and the activity was associated with aggregate-sized material. Further, heat-aggregated myeloma proteins were active, providing compelling evidence that the activity of the LPS was expressed via enhanced platelet sensitivity to IgG aggregates, and not through antilipid A antibodies or trace contaminants of the protein.

Aggregated IgG stimulates both platelet (1) and neutrophil (23) secretion. In the latter case, small soluble aggregates have been reported to be virtually inactive, and this activity is enhanced markedly when the size of the aggregates are increased or they are bound to surfaces (23). In contrast to the neutrophil, we have shown that platelet secretion is stimulated by very small soluble IgG aggregates indicating a difference in the manner in which these cell types respond to immune aggregates. The effects of both large and small IgG aggregates and insoluble immune complexes on platelets were enhanced by LPS R595. This indicates that although LPS R595 binds immune aggregates, the enhancement of cell stimulation is not due to a simple increase in the valence of the stimulus as a result of being enlarged or insolubilized.

The ability of immune aggregates to bind and activate C_1 and to activate

[3] M. H. Ginsberg and P. M. Henson. Manuscript in preparation.

platelets, the inhibition of IgG aggregate-induced platelet stimulation by C_1 (4), and the reported presence of C_1 activity on washed platelets (8) has led to the hypothesis that C_1 is a platelet immunoglobulin receptor (8). Lipid A or LPS R595, potent activators of C_1 (12, 13), had little platelet stimulating activity themselves. This indicates that a molecule's ability to activate C_1 does not always coincide with the ability to initiate the release reaction of washed human platelets. The interaction of platelet-bound C_1 with LPS R595 or lipid A might have played a role in the enhancement of platelet response to immune aggregates, but the data presented here do not permit one to favor this possibility above a wealth of others. What is clear is that these two potent activators of C_1 are not potent platelet stimuli and that IgG_4 aggregates, which do not activate C_1, stimulate the release reaction (4, 5). This implies different specificities between the Fc recognition unit of complement, C_1, and the postulated platelet Fc receptor.

Bacterial LPS may differ widely in biologic properties. One important determinant of these differences is the relative content of lipid A and polysaccharide (9). Thus, polysaccharide-rich LPS activates primarily the alternative pathway of complement, whereas lipid A-rich LPS activates primarily the classical pathway (12, 13). Mueller-Eckhardt and Lüscher (24) have reported that LPS from *Escherichia coli* 055:B5 failed to stimulate washed human platelets in the presence of a gamma globulin preparation. We have had similar results with phenol extracted (15) polysaccharide-rich LPS from *E. coli* 055:B5.[4] Because *E. coli* O55:B5 LPS contains a typical lipid A moiety, these data underscore modulation of lipid A effects by the polysaccharide constituent of LPS and the importance of using well-characterized LPS preparations in defining specific LPS effects.

LPS is capable of initiating a wide variety of injurious responses in vivo. This may be a result of its ability to activate a number of mediation systems. Recently, it has become clear that the common lipid region of LPS alone may provide the LPS activation signal to many cells, such as murine B cells (25–28) and macrophages,[5] and human procoagulant activity generating leukocytes (29) and to humoral systems such as complement via C_1 (12, 13). Strikingly, immune complexes have important effects on each of these systems as well (3, 30, 31) and initiate a wide variety of injurious responses in vivo. The observation that the common lipid A moiety of LPS enhances a cell's response to immune complexes may then provide an important clue to the mechanism of the multiplicity of LPS effects in vitro and in vivo.

Summary

The effect of the common lipid moiety of bacterial LPS on secretion from washed human platelets has been studied. The lipid A-rich LPS of *S. minnesota* R595 and a lipid A preparation both potentiated platelet serotonin secretion in response to IgG aggregates or immune complexes up to 50-fold but had little

[4] M. H. Ginsberg and D. C. Morrison. Manuscript in preparation.

[5] W. F. Doe, S. T. Yang, D. C. Morrison, S. J. Betz, and P. M. Henson. Macrophage stimulation by bacterial lipopolysaccharides. II. Evidence for independent stimulation signals delivered by lipid A and by a protein-rich fraction of LPS. Submitted for publication.

effect in the absence of IgG. Lipid A has been shown to bind immune aggregates, raising the possibility that its mechanism of action involved effective enlargement or insolubilization of the aggregates. IgG aggregates of dimer to tetramer size were shown to be platelet stimuli, equivalent on a weight basis to larger soluble aggregates. The effect of both sizes of aggregates on platelets were equally enhanced by the LPS, indicating that increased size of aggregates alone could not account for the effect of LPS. Similarly, because lipid A-rich LPS enhanced platelet response to already insoluble immune complexes, its mechanism of action cannot simply be insolubilization of immune aggregates. These LPS did not enhance platelet stimulation by antiplatelet antibody, monosodium urate crystals, or thrombin and only slightly enhanced stimulation by insoluble human skin collagen. This indicates some stimulus specificity in the ability of LPS to increase platelet secretion. The enhancement of cell response to immune complexes by the common lipid region of LPS may represent a mechanism for the diverse effects of LPS in vivo and in vitro.

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