Mobilization of Potent Plasma Bactericidal Activity during Systemic Bacterial Challenge

Role of Group IIA Phospholipase A_2

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Abstract

Extracellular mobilization of Group IIA 14-kD phospholipase A_2 (PLA_2) in glycogen-induced rabbit inflammatory peritoneal exudates is responsible for the potent bactericidal activity of the inflammatory fluid toward *Staphylococcus aureus* (1996. *J. Clin. Invest.* 97:250–257). Because similar levels of PLA_2 are induced in plasma during systemic inflammation, we have tested whether this gives rise to plasma bactericidal activity not present in resting animals. Baboons were injected intravenously (i.v.) with a lethal dose of *Escherichia coli* and plasma or serum was collected before and at hourly intervals after injection. After infusion of bacteria, PLA_2 levels in plasma and serum rose $>$ 100-fold over 24 h to $\sim$ 1 μg PLA_2/ml. Serum collected at 24 h possessed potent bactericidal activity toward *S. aureus, Streptococcus pyogenes,* and encapsulated *E. coli* not exhibited by serum collected from unchallenged animals. Bactericidal activity toward *S. aureus* and *S. pyogenes* was nearly completely blocked by a monoclonal antibody to human Group IIA PLA_2 and addition of purified human Group IIA PLA_2 to prechallenge serum conferred potent antistaphylococcal and antistreptococcal activity equal to that of the 24 h postchallenge serum. PLA_2–dependent bactericidal activity was enhanced $\sim$ 10$\times$ by factor(s) present constitutively in serum or plasma. Bactericidal activity toward encapsulated *E. coli* was accompanied by extensive bacterial phospholipid degradation mediated, at least in part, by the mobilized Group IIA PLA_2 but depended on the action of other bactericidal factors in the 24-h serum. These findings further demonstrate the contribution of Group IIA PLA_2 to the antibacterial potency of biological fluids and suggest that mobilization of this enzyme during inflammation may play an important role in host defense against invading bacteria.

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Introduction

Group IIA phospholipase A_2 (PLA_2)[1] represents one of several isoforms of 14-kD PLA_2 expressed in mammals (1, 2). It is produced by many different cell types including phagocytic cells, platelets, Paneth cells, and lacrimal glands (1, 2–4). The extracellular levels of Group IIA PLA_2 can be appreciable in certain body compartments (4–6). However, levels of this enzyme in plasma or many other extracellular fluids are normally very low ($<$ 10 ng/ml). Production and secretion of Group IIA PLA_2 is markedly increased during inflammation (3, 6) and as a result, extracellular levels of this enzyme in the circulation and at sites of inflammation may reach levels that are 100–1,000 times greater than normal resting concentrations (i.e., 0.1–1.0 μg/ml).

This upregulation of Group IIA PLA_2 has been known for many years. Nevertheless, the functional consequences of increased extracellular levels of Group IIA PLA_2, whether beneficial and/or harmful, remains uncertain (7). Because mobilization of host defenses is an essential function of inflammation, we have examined the possible contribution of inflammatory fluid Group IIA PLA_2 to antibacterial defenses. We have demonstrated that at enzyme concentrations present at inflammatory sites both rabbit and human Group IIA PLA_2 can, in concert with other host defense systems, increase destruction of Gram-negative bacteria (8–11) and by itself, kill many Gram-positive bacteria (12). In contrast, Group I (e.g., pancreatic) PLA_2 displays little or no activity toward these bacterial targets (9, 11–13). Thus, the antibacterial activity of Group IIA PLA_2 appears to be a specific attribute of this mammalian 14-kD isoform.

In an experimentally induced local inflammatory (ascitic) fluid in rabbits, the mobilization of Group IIA PLA_2 is fully responsible for the potent bactericidal activity expressed by this fluid toward *Staphylococcus aureus* and several other Gram-positive bacteria (12). Normal plasma, by contrast, contains little PLA_2 or antistaphylococcal activity. Addition to plasma of unchallenged animals of purified rabbit or human Group IIA PLA_2, but not Group I pancreatic PLA_2, confers potent bactericidal activity toward *S. aureus.* These findings suggest that mobilization of Group IIA PLA_2 in plasma during systemic inflammatory responses could yield systemic extracellular bactericidal activity not present at rest.

To test this hypothesis, we have in this study measured the PLA_2 and antibacterial activities of serum collected serially

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1. *Abbreviation used in this paper:* PLA_2, phospholipase A_2.

from baboons before and after intravenous infusion of a lethal inoculum of *Escherichia coli.* We show that the host response to this systemic bacterial challenge includes mobilization of broad spectrum bactericidal activity contributed in large part by the action of the Group IIA PLA_2.

Methods

Bacteria. Bacteria used in this study included *S. aureus* 502A, a random clinical isolate of *Streptococcus pyogenes* (part of the collection of the Department of Microbiology, New York University Medical School, New York) and K1-encapsulated *E. coli* strains O7K1, an isolate from a case of human bacteremia, and K1/r, isolated from a urinary tract infection. To facilitate assay of the phospholipolytic action of Group IIA PLA_2 during incubation of encapsulated *E. coli* in serum, we created a phospholipase-deficient (*pldA*$^-$) derivative of *E. coli* K1/r by P1-transduction of pldA::kan^r from *E. coli* PC 2154 pldA::kan^r (14). All bacteria were grown in trypticase soy broth at 37°C overnight, washed once, and diluted 1:100 in fresh media and subcultured to mid-to-late logarithmic phase ($\sim$ 3 h). After harvesting, the bacteria were sedimented by centrifugation at 14,000 *g* for 5 min and resuspended in sterile physiological saline at a concentration of 10^9 bacteria/ml.

Collection of baboon plasma and serum. Arterial blood samples were drawn from animals just before and at timed intervals after intravenous infusion of *E. coli* (O86; 10^{10} cfu/kg in 30 min) to induce (lethal) sepsis (15). Plasma was obtained from blood collected with buffered citrate as an anticoagulant and serum from blood collected without anticoagulants; both were stored at −70°C until use. At the doses used for anticoagulation, heparin and to a lesser extent citrate, inhibit the activity of Group IIA PLA_2 (12, 16). Therefore, serum was used for measurements of antibacterial activity. However, because of the much greater sensitivity of assays of PLA_2 enzymatic activity against autoclaved *E. coli* (17), these assays could be carried out equally well with diluted serum or plasma (i.e., inhibitory effect of citrate is diluted out). Cationic proteins (including Group IIA PLA_2) were removed quantitatively from serum or plasma by adsorption to CM-Sephadex resin (0.1 ml resin equilibrated with 10 mM Tris-buffered physiological saline, pH 7.4/ml of plasma or serum) (18); the recovered flow-through fraction contained > 99% of the original serum (plasma) protein but no Group IIA PLA_2.

Collection of human plasma and serum. Peripheral blood was collected from healthy human volunteers after informed consent either into tubes containing buffered citrate for separation of plasma or tubes without anticoagulant for collection of serum. To prepare a protein-depleted fraction of serum that retains the electrolyte composition of the biological fluid, serum was subjected to ultrafiltration (Centricon-10; Amicon, Inc., Danvers, MA) followed by sterile filtration of the resulting filtrate. The recovered ultrafiltrate contained < 0.2% of the total protein content by OD_{280} of serum. For bioassays, the ultrafiltrate was supplemented with 40 mg/ml human serum albumin to match the albumin content of serum or plasma. In assays comparing the PLA_2-enhancing activity in serum and plasma, serum was supplemented with buffered citrate.

PLA_2. Recombinant human Group IIA PLA_2 was expressed and purified as described previously (9). Protein concentrations of purified PLA_2 preparations were determined by OD_{280} using the known extinction coefficient of this protein.

Assay of PLA_2 activity. PLA_2 activity of various collections of serum or plasma was measured by assay against [1-^{14}C]oleate-labeled autoclaved *E. coli* as described previously (12, 17). One arbitrary unit of PLA_2 activity is defined as hydrolysis of 1% of bacterial substrate/h, corresponding to 50 pmol of phospholipid degraded/h. The specific activity of rabbit and human Group IIA and pig Group I PLA_2 toward this substrate is $\sim$ 200,000 U/μg protein (9, 13).

Assay of bactericidal activity. An effect of serum or purified recombinant human Group IIA PLA_2 on bacterial viability was determined by measuring bacterial colony-forming ability after incubation for 2 h at 37°C. Typical incubation mixtures contained 2×10^5 bacteria in a total volume of 100 μl in which buffered plasma, serum or serum ultrafiltrate represented 90% of the reaction volume. The buffer used was either 20 mM HEPES or, when plasma was used, buffered citrate, both at pH 7.4. At the end of the incubation, aliquots of the suspensions were taken, serially diluted in sterile physiological saline, and transferred to 5 ml of molten (48–50°C) 1.3% (wt/vol) Bacto-agar (Difco Laboratories Inc., Detroit, MI) containing 3% (wt/vol) trypticase soy broth. Bacterial viability was measured as the number of colonies formed after incubation at 37°C for 18–24 h.

Radiolabeling of bacterial phospholipids during growth. To uniformly label the lipids of *S. aureus* and *E. coli* K1/r (pldA::kan^r), the bacteria were grown in subculture at 37°C in nutrient broth supplemented with 2.5 μCi/ml of [1-^{14}C]oleic acid (56 mCi/mmol; DuPont New England Nuclear, Boston, MA) and 0.03% (wt/vol) bovine serum albumin. Bacteria were grown to mid-log phase, washed, resuspended in fresh media without oleic acid, incubated at 37°C for 20–30 min, and washed in media supplemented with 0.5% (wt/vol) albumin before use. Approximately 70% of the oleate-labeled material was phospholipid (mainly phosphatidylglycerol; [reference 12]) in *S. aureus* and essentially all was labeled phospholipid in *E. coli* (17).

Assay of degradation of bacterial phospholipids during incubation with serum (PLA_2). [1-^{14}C]Oleate-prelabeled bacteria ($\sim$ 2,500 cpm/10^6 bacteria) were incubated with or without serum or purified PLA_2 in the standard incubation mixture except that 5×10^6 *S. aureus*/0.5

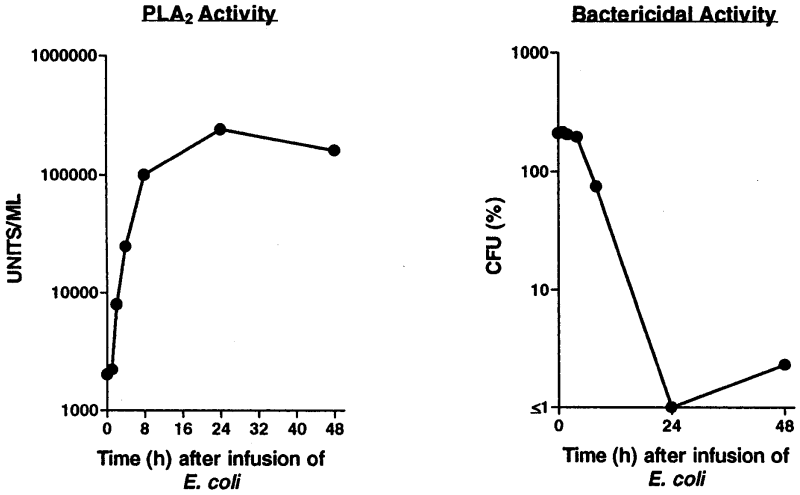

Figure 1. Effect of i.v. infusion of a lethal inoculum of *E. coli* on PLA_2 (*left*) and antistaphylococcal (*right*) activities of baboon serum. Serum PLA_2 activity against autoclaved *E. coli* and bactericidal activity toward *S. aureus* were measured as described in Methods. Bacterial viability of incubated samples is expressed as the percentage of viability of the initial bacterial inoculum. The data shown represent the mean of the activities of sera collected from four different animals, each measured in duplicate.

Table I. PLA_2 Accumulating in Baboon Plasma (Serum) during Systemic Bacterial Challenge Is Blocked by a Group IIA–specific Inhibitor (LY311727)

Sample	Autoclaved *E. coli* PL Degradation (%)*	
	−LY311727	+LY311727 (10 μM)
Human Group IIA PLA_2 (1 ng)	25.2±2.4	0.3±0.2
Pig Group I PLA_2 (1 ng)	24.4±1.3	25.1±2.1
Baboon serum‡ (1 μl)	22.1±2.7	0.2±0.1

*Incubations were carried out at 37°C for 10 min with 2×10^8 radiolabeled autoclaved *E. coli.* PL degradation was measured as described in Methods. Results shown represent the mean±SEM of three independent determinations. ‡Serum collected 24 h after infusion of *E. coli* as described in Methods. PLA_2 activity of plasma collected at this time was essentially the same as that of serum.

ml or 5×10^6 *E. coli* K1/r/0.05 ml were used. Bacterial phospholipid degradation was measured as the accumulation of radioactivity in the extracellular medium (free fatty acids and lyso compounds are quantitatively recovered in the medium complexed with serum albumin whereas remaining undegraded phospholipids remain in the bacterial pellet; [12, 17]) and confirmed by extraction and thin-layer chromatographic analysis (11, 12).

Assay of inhibitory effect of inhibitors of human (mammalian) Group IIA PLA_2. Two inhibitors were used: (i) a neutralizing mouse mAb raised against human Group IIA PLA_2 (IgG_{2a} [184]; 200 μg/ml; [reference 19]); (ii) an active-site directed, indomethacin-related, inhibitor (LY311727) with apparent specificity for mammalian Group IIA PLA_2 (20). The latter compound was dissolved at 10 mM in DMSO and used at a final concentration of 10 μM. PLA_2-containing samples were preincubated for 30 min at room temperature with either of these inhibitors before addition of live or autoclaved bacterial targets. Preincubation of PLA_2-containing samples with isotype-matched unrelated mAb (nonneutralizing mAb [2–4] to the bactericidal/permeability-increasing protein; obtained from XOMA Corp., Berkeley, CA) or DMSO (0.1%, vol/vol) alone had no effect on PLA_2 activity.

Results

Mobilization of PLA_2 and antistaphylococcal activities in baboon sera (plasma) after systemic bacterial challenge. Plasma and serum collected from unchallenged baboons contained low levels of PLA_2 activity (< 3,000 U/ml using autoclaved *E. coli* as substrate) and no detectable antibacterial activity toward *S. aureus* (Fig. 1). After intravenous infusion of a lethal inoculum of *E. coli*, serum levels of PLA_2 rose dramatically as previously reported (15), reaching a maximum of 200,000–300,000 U/ml (~ 1 μg/ml) at 24 h after induction of systemic inflammation. This activity was completely inhibited by LY311727 (Table I), an active site–directed inhibitor with specificity for Group IIA PLA_2 (Table I; [reference 20]) suggesting that the enzyme mobilized in serum is a baboon Group IIA homologue. Virtually identical results were obtained when plasma rather than serum was collected from the animals (data not shown). In parallel to the increase in PLA_2 levels, serum acquired potent bactericidal activity toward *S. aureus* (Fig. 1). Serum collected at 24 h after infusion of *E. coli* was able to kill > 3 logs of 10^6 bacteria/ml during 2-h incubation.

Role of Group IIA PLA_2 in bactericidal activity of baboon serum vs S. aureus after systemic bacterial challenge. To determine the role of Group IIA PLA_2 in the bactericidal activity exhibited by baboon serum toward *S. aureus* after systemic bacterial challenge, the antibacterial activity of baboon serum collected 24 h after infusion was measured in the presence or absence of a neutralizing mAb to human Group IIA PLA_2. For comparison, similar assays were carried out with recombinant purified human Group IIA PLA_2 diluted in serum collected from unchallenged animals. When expressed in relation to the (estimated) amount of PLA_2 added, the bactericidal activity of purified human Group IIA PLA_2 and of whole baboon 24 h serum toward *S. aureus* was closely similar (Fig. 2). Moreover, the antistaphylococcal activity expressed by baboon serum collected 24 h (or 48 h; data not shown) after infusion was nearly completely blocked by a neutralizing mAb raised against human Group IIA PLA_2 (Fig. 2). These findings demonstrate that the serum bactericidal activity induced by systemic bacterial challenge is principally due to the mobilization of Group IIA PLA_2.

Constitutive presence in plasma of factor(s) that enhance the antistaphylococcal activity of Group IIA PLA_2. In the experiments shown in Fig. 2, purified PLA_2 or the 24-h baboon serum was diluted in serum collected from unchallenged animals to provide assay conditions that mimicked as closely as possible the extracellular environment in which the PLA_2 accumu-

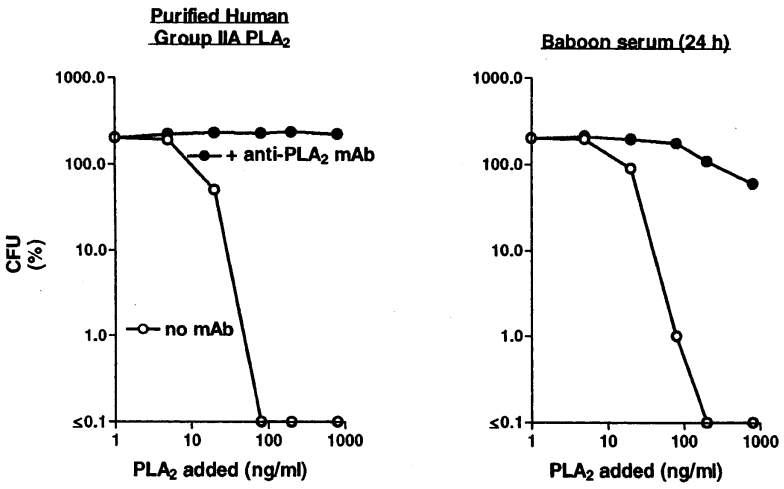

Figure 2. Dose-dependent bactericidal activity toward *S. aureus* of purified human Group IIA PLA_2 (*left*) and baboon serum collected 24 h after infusion of *E. coli* (*right*) in the presence and absence of anti–human Group IIA mAb (10 μg/ml). The highest dose of serum tested corresponded to 90% (vol/vol) serum. Dilutions of human PLA_2 and baboon serum were in HEPES-buffered resting serum (*human* or *baboon*) so that all samples contained 90% serum buffered with 20 mM HEPES, pH 7.4. Serum or PLA_2 in serum were preincubated±mAb for 30 min at room temperature before adding the bacteria. Addition of 10 μg/ml of isotype-matched irrelevant antibody had no effect on the bactericidal activity of either purified human PLA_2 or whole baboon serum. The results shown represent the mean of three independent determinations.

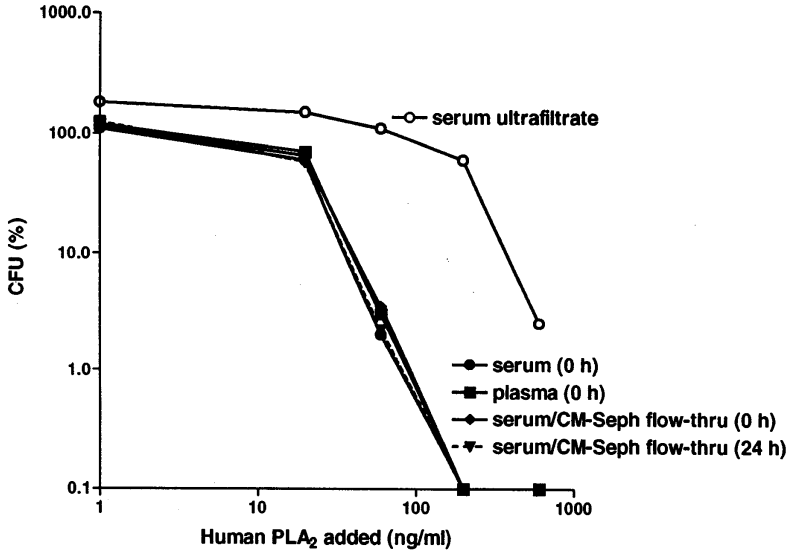

Figure 3. Plasma (*serum*) enhances the bactericidal activity of human Group IIA PLA_2 toward *S. aureus.* Incubations were carried out in the indicated media (90%, vol/vol) supplemented with buffered citrate, pH 7.4. The same results were obtained with serum-derived fractions in the absence of citrate except that PLA_2 dose curves were shifted two- to threefold to the left. *CM-Seph. flow-thru* corresponds to serum fractions obtained after adsorption of cationic proteins including Group IIA PLA_2 to CM-Sephadex as described in Methods. The results shown represent the mean of three or more experiments.

lated. To test whether plasma or serum contains factors that modulate PLA_2 activity toward *S. aureus*, the bactericidal potency of added purified Group IIA PLA_2 was measured in the presence of serum, plasma, or a protein-depleted ultrafiltrate of serum (see Methods). Fig. 3 shows that the bactericidal potency of human (or rabbit; data not shown) Group IIA PLA_2 was $\sim$ 10 times greater in serum or plasma than in the protein-poor serum ultrafiltrate (supplemented with 40 mg/ml albumin to match the levels of that protein present in serum/plasma). The same potentiating effect was seen with human and baboon sera before and after heat treatment (56°C for 45 min) to inactivate complement (data not shown). Serum depleted of cationic proteins by adsorption to CM-Sephadex also retained full potentiating activity (Fig. 3). After removal of PLA_2 by adsorption to CM-Sephadex, baboon sera collected just before and 24 h after infusion of *E. coli* similarly potentiated the bactericidal activity of added human Group IIA PLA_2 toward *S. aureus.* Thus, plasma contains factor(s) that enhance the antistaphylococcal activity of Group IIA PLA_2. In contrast to the enzyme itself, PLA_2 potentiating factor(s) are apparently constitutively present in plasma.

Potentiating effects of serum are restricted to antibacterial activity of Group IIA PLA_2. Bacterial killing by Group IIA PLA_2 is accompanied by virtually quantitative degradation of bacterial membrane phospholipids (12). We therefore examined whether the potentiating effects of serum on the antistaphylococcal activity of Group IIA PLA_2 include an increase in bacterial phospholipid degradation. Fig. 4 shows that the greater bactericidal potency of Group IIA PLA_2 in serum was paralleled by increased bacterial phospholipid degradation. In contrast, the enzymatic activity of this enzyme toward artificial presentations of the bacterial phospholipid, either autoclaved bacteria (Fig. 4) or extracted lipid dispersions (data not shown), was the same in serum and the serum ultrafiltrate. Thus, the potentiating effects of serum (plasma) on Group IIA PLA_2 activity appear to be specific for the action of the enzyme on intact bacteria.

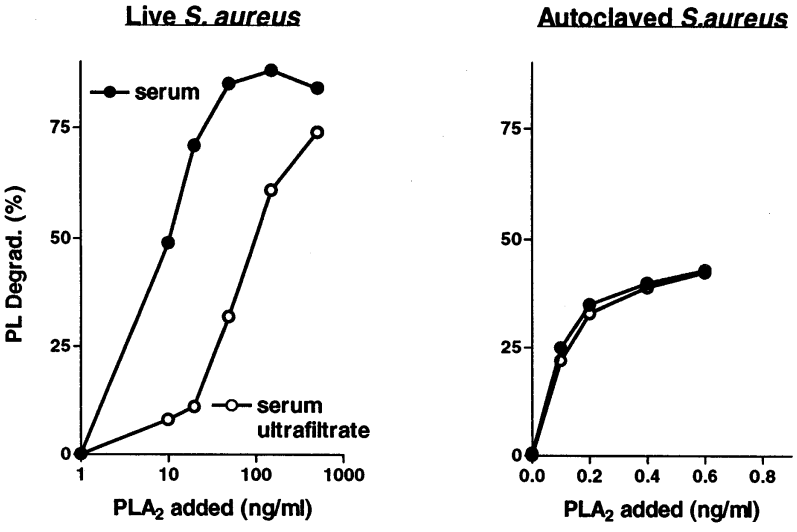

Figure 4. Serum stimulates phospholipolytic action of human Group IIA PLA_2 toward intact *S. aureus* (*left*) but not toward autoclaved *S. aureus* (*right*). Incubations were carried out for 1 h at 37°C with 5×10^6 (*intact* or *autoclaved*) bacteria/ml in the indicated medium (90%, vol/vol) buffered with 20 mM HEPES, pH 7.4. The results shown represent the mean of the three separate determinations.

Increased bactericidal activity of 24-h baboon serum toward other invasive bacterial pathogens: role of Group IIA PLA_2. Increased serum bactericidal activity, induced by systemic inflammation, is manifest not only toward *S. aureus* but also against other important invasive bacterial pathogens such as Group A *streptococci* and K1-encapsulated *E. coli.* Against each of these bacteria, serum collected 24 h after infusion of *E. coli* exhibited potent bactericidal activity not present in serum collected before systemic bacterial challenge (Table II). Addition of purified human Group IIA PLA_2 to resting state serum, at concentrations present in serum 24 h after bacterial infusion, reproduced the bactericidal activity of the 24 h serum toward the Gram-positive bacteria *S. aureus* and *S. pyogenes*, but not toward *E. coli* O7K1 or K1/r (Table II). Conversely, a neutralizing mAb to human Group IIA PLA_2 substantially inhibited the bactericidal activity of the 24-h baboon serum toward *S. aureus* and *S. pyogenes*, but not toward K1-encapsulated *E. coli.* Thus, the potent antistaphylococcal and antistreptococcal activity appearing in serum during a systemic inflammatory response reflects mainly the accumulation of Group IIA PLA_2. In contrast, the increased bactericidal activity toward encapsulated *E. coli* in the 24-h serum is not directly attributable to the PLA_2 but reflects the accumulation of other antibacterial agents that also facilitate phospholipolysis. This action is partially inhibited by the anti-PLA_2 mAb (Table II) demonstrating that the Group IIA PLA_2 also contributes to the destruction of these Gram-negative bacteria.

Discussion

We have shown that the accumulation of Group IIA PLA_2 in the circulation of baboons during a systemic inflammatory response results in the expression in plasma of potent bactericidal activity toward *S. aureus* and other potentially invasive Gram-positive bacterial pathogens. Identification of the presence and biologic role of this enzyme in plasma has relied, in part, on the use of a neutralizing mAb raised against human Group IIA PLA_2 with previously documented specificity for this isoform (19). That the mobilization of phospholipase activity is restricted to this low molecular weight PLA_2 isoform under these experimental conditions is consistent with previous studies (15) and has been confirmed by the inhibitory effects of an indomethacin-related compound (Table I) with apparent specificity for Group IIA PLA_2 (Table I; [reference 20]). The closely similar bactericidal activities toward *S. aureus* (and *S. pyogenes*) of baboon serum collected 24 h after i.v. administration of *E. coli* and of purified human Group IIA PLA_2 added to prechallenge serum (Fig. 2, Table II) are also consistent with a prominent role of this isoform in the activity of the baboon serum. The residual activity expressed by the 24-h baboon serum in the presence of the neutralizing mAb (Fig. 2, Table II) may reflect the mobilization of other (weaker) antibacterial agents during the systemic inflammatory response or simply incomplete inhibition of the baboon Group IIA PLA_2, present at high concentrations in the 24-h serum, by the anti–human Group IIA PLA_2 mAb. The incomplete inhibition by this mAb of the phospholipid degradation of phospholipase-deficient *E. coli* K1/r produced by the 24-h serum (Table II) seems most consistent with the latter possibility.

In contrast to rabbit and rat sera (3, 12, 21, 22), human and baboon sera from unchallenged animals contain little PLA_2 (and antistaphylococcal) activity (Fig. 1; Table II; [21, 22]). Preliminary experiments suggest that this may reflect differences among these species in secretion of Group IIA PLA_2 from platelets during in vitro cell activation (Dominiecki, M., M. Yeaman, and J. Weiss, unpublished observations). The low basal levels of PLA_2 and antistaphylococcal activities in baboon serum made it possible to monitor changes in circulating levels of PLA_2 and antibacterial activities during systemic inflammation by assays of serum. Thus, we could avoid the use of anticoagulants that can interfere with expression of Group IIA PLA_2 activity (12, 16). Because enzymatic assays using autoclaved *E. coli* are so sensitive (activity can be detected in as little as 1 μl of plasma from unchallenged animals; [17]), PLA_2 activity could be measured in plasma (see Methods) as well as serum to confirm that the time-dependent mobilization of extracellular Group IIA PLA_2 activity occurred in vivo and not during in vitro clotting. The kinetics of PLA_2 accumulation in plasma that we observed are consistent with the view that the Group IIA enzyme is an acute phase reactant (23). The cellular source(s) of the secreted enzyme under these conditions remains to be determined.

The potent bactericidal activity toward *S. aureus* expressed in plasma during the systemic inflammatory response reflects the mobilization of relatively high concentrations of Group IIA PLA_2, the intrinsic potency of this enzyme and also the presence in plasma of factor(s) that further enhance the bactericidal potency of the PLA_2 (Fig. 3). In contrast to the PLA_2 it-

Table II. Antibacterial Spectrum of Baboon Serum Collected after Systemic Bacterial Challenge: Role of Group IIA PLA_2

Treatment	*S. aureus*	*S. pyogenes*	*E. coli* O7K1	*E. coli* K1/r	
	CFU; %	*CFU; %*	*CFU; %*	*CFU; %*	*PL degradation; %*
Serum (0 h)	190	220	130	68	13
Serum (0 h) + purified human Group IIA PLA_2	< 0.1	3	120	60	47
Serum (24 h)	< 0.1	4	< 0.1	< 0.1	78
Serum (24 h) + antihuman Group IIA PLA_2 mAb	70	120	< 0.1	< 0.1	44

Bacteria (*S. aureus*, *S. pyogenes*, and *E. coli* O7K1 at 10^6/ml, ^{14}C-oleate prelabeled *E. coli* K1/r at 10^8/ml) were incubated with 90% (vol/vol) serum buffered with 20 mM HEPES, pH 7.4, for 2 h before measurement of bacterial viability and phospholipid (*PL*) degradation as described in Methods. The viability of treated bacteria is expressed as percentage of viability of the initial bacterial inoculum and PL degradation is expressed as the percentage of total labeled bacterial lipids degraded. The results shown represent the mean of at least two closely similar experiments. Serum was preincubated with the anti-PLA_2 mAb (200 μg/ml) 30 min before addition of bacteria. Preincubation of serum with an isotype-matched unrelated control mAb had no effect on serum activities.

self, these factor(s) are noncationic in nature and present constitutively in plasma (human, baboon, or rabbit, Fig. 3; data not shown). The PLA_2-enhancing activity of plasma potentiates specifically the action of the Group IIA enzyme against live (intact) bacterial targets suggesting the action of agent(s) that modify the bacterial cell wall and thereby facilitate enzyme access and action against cell membrane phospholipids.

We have previously demonstrated that the neutrophil-derived bactericidal/permeability–increasing protein and (sublethal) assemblies of the membrane–attack complex of complement promote Group IIA PLA_2 action against Gram-negative bacteria by producing sublethal alterations of the outer envelope of these bacteria (10, 11, 24). Thus, the potentiation of Group IIA PLA_2 action toward Gram-positive bacteria by noncationic and heat-stable elements of plasma is reminiscent of the actions of other (i.e., cationic and heat-labile) host PLA_2-potentiating factors acting specifically against Gram-negative bacteria. However, the antibacterial role of the Group IIA PLA_2 against Gram-positive and Gram-negative bacteria is very different. In inflammatory fluids of either intravascular (Table II) or extravascular (12, 18) origin, the Group IIA PLA_2 is the primary bactericidal agent against many Gram-positive bacteria and its potency may be enhanced by other host (plasma) factors. In contrast, at the concentrations present in these fluids, this enzyme alone has no independent antibacterial activity against Gram-negative bacteria and does not degrade the bacterial phospholipids. Its contribution to the destruction of these bacteria depends entirely on the action of other antibacterial host factors. The greater phospholipid degradation produced by the 24-h serum vs that produced by resting serum simply supplemented with purified Group IIA PLA_2 (Table II) is consistent with the role of other antibacterial agents (mobilized during the systemic inflammatory response) in promoting PLA_2 action against these bacteria. Under these potently cytotoxic conditions, very little Group IIA PLA_2 may be needed to produce extensive bacterial phospholipid degradation (11), presumably explaining the appreciable phospholipid degradation still produced in the presence of the anti-PLA_2 mAb.

In summary, our findings extend previous observations strongly suggesting a physiological role in host defense against microbial invaders for the extracellular mobilization of Group IIA PLA_2 during inflammation. Thus, in primates as well as rabbits, during both local and systemic inflammatory responses triggered by either sterile irritants or bacterial inocula, the accumulation of extracellular Group IIA PLA_2 is largely responsible for the expression in inflammatory fluids of potent bactericidal activity toward many Gram-positive bacterial pathogens and contributes to the destructive power of these fluids toward complement and phagocytosis-resistant Gram-negative bacteria. It remains to be determined whether under conditions of self-limiting infection, the rate and extent of mobilization of Group IIA PLA_2 is sufficient to contribute significantly to the eradication of these invading microorganisms.

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