Effect of d-Alanylation of (Lipo)Teichoic Acids of *Staphylococcus aureus* on Host Secretory Phospholipase A2 Action before and after Phagocytosis by Human Neutrophils

Catherine L. Hunt, William M. Nauseef and Jerrold P. Weiss

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1 Address correspondence and reprint requests to Dr. Jerrold P. Weiss, Inflammation Program, 2501 Crosspark Road, Coralville, IA 52241. E-mail address: jerkrold-weiss@uiowa.edu

2 Abbreviations used in this paper: PMN, polymorphonuclear leukocyte; CL, cardiolipin; gIIA PLA2, group IIA phospholipase A2; gV PLA2, group V phospholipase A2; gX PLA2, group X phospholipase A2; HSA, human serum albumin; PL, phospholipid; PG, phosphatidylglycerol; wt, wild type.

Catherine L. Hunt,*† William M. Nauseef,*‡ and Jerrold P. Weiss2*†‡

Intracellular antibacterial proteins (e.g., group IIA phospholipase A2 (gIIA PLA2)). Accumulation of gIIA PLA2 in inflammatory fluids confers potent extracellular antistaphylococcal activity and at lower concentrations promotes bacterial phospholipid degradation during phagocytosis of S. aureus by human neutrophils. D-alanylation of (lipo)teichoic acids of S. aureus increases bacterial resistance to gIIA PLA2 ~100-fold, raising the possibility that the resistance of ingested S. aureus to related gV and gX secretory PLA2 present in human neutrophil granules depends on D-alanylation mediated by the dlt operon. However, we show that isogenic wild-type and dltA S. aureus are equally resistant to gV/X PLA2 during phagocytosis and when exposed to the purified enzymes. The fates of wild-type and dltA S. aureus exposed to serum and human neutrophils differed significantly only when extracellular gIIA PLA2 was also present before phagocytosis. The extreme potency of the gIIA PLA2 toward dltA S. aureus suggests that even small amounts of this extracellular enzyme mobilized early in inflammation could contribute substantially to the overall cytotoxicity of acute inflammatory exudates toward S. aureus when D-alanylation of (lipo)teichoic acids is limiting. The Journal of Immunology, 2006, 176: 4987–4994.

The fates of wild-type and dltA S. aureus

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Therefore, in this study we compared the sensitivity of ingested wt and dltA S. aureus to the cytotoxic effects of human neutrophils, including the actions of gV and gX PLA2. In contrast to effects of α-alanylation on the antiphagococcal activity of gIA PLA2, there was little or no effect of α-alanylation of S. aureus (lipo)teichoic acids on serum (complement)-mediated opsonization of the bacteria, intracellular killing by neutrophils, or the actions of gV/X PLA2. However, substantial differences in the fate of wt or dltA S. aureus exposed to neutrophils were seen when the bacteria were exposed to extracellular gIA PLA2 before phagocytosis. Thus, the effects of α-alanylation on resistance of S. aureus to innate human host defenses appear to principally reflect alterations in susceptibility to the action of gIA PLA2. The extreme potency of this enzyme toward dltA S. aureus suggests that even small amounts of extracellular enzyme mobilized early in the host response could contribute substantially to the overall cytotoxicity of acute inflammatory exudates toward S. aureus when α-alanylation of (lipo)teichoic acids is limiting.

Materials and Methods

Materials

Tryptic soy broth and Bacto Agar were purchased from BD Biosciences. HEPES and HBSS (HBSS1) or without (HBSS2) divalent cations were obtained from Mediatech CellGro. Clinical grade dextran 500 was purchased from PerkinElmer. Chloroform, methanol, glacial acetic acid, and the HEMA 3 stain kit were purchased from Fisher Scientific. Silica gel 60 high performance TLC plates were purchased from EMD Chemicals. Purified lipid standards for phosphatidylglycerol [PG; 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] and cardiolipin (CL; tetraoleoyl cardiolipin), lysophosphatidylglycerol [1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-rac-(1-glycerol)], monolysocardiolipin (purified from bovine heart; 92% linoleic), and dilyso cardiolipin (purified from bovine heart; 96% linoleic) were purchased from Avanti Polar Lipids.

Bacteria

Isogenic dltA S. aureus (SA113) and dltA S. aureus were provided by A. Peschel (University of Tubingen, Tubingen, Germany) (28). The bacteria were grown overnight in tryptic soy broth at 37°C, diluted to an OD550 of 0.05 in fresh medium, and subcultured for 2–2.5 h to midlogarithmic phase. Subcultured bacteria were washed once, resuspended in 20 mM HEPES-buffered HBSS (pH 7.4), and used immediately for opsonization (see below). Bacterial concentrations were monitored by absorbance at 550 nm and were verified by counting bacteria with a hemocytometer and measuring CFU after overnight incubation at 37°C in tryptic soy agar. For each strain, 1 CFU corresponded to approximately three or four cocci.

Bacterial lipid labeling with [14C]oleic acid

Bacterial subcultures were supplemented with 1 μCi/ml [1-14C]oleic acid and 0.01% (w/v) HSA. After 2-h incubation, S. aureus were pelleted by centrifugation for 5 min at ~5000 × g, resuspended in 0.5 vol of fresh medium without radiolabel, and incubated an additional 30 min at 37°C. HSA was then added to a final concentration of 0.5%, and the bacteria were pelleted by centrifugation to remove any remaining unincorporated [1-14C]oleic acid in the supernatant as complexes with albumin. The sedimented bacteria were resuspended at room temperature at a concentration of 107 CFU/ml in 20 mM HEPES-buffered HBSS and were used within 30 min for opsonization.

PLA2

Purified recombinant human gIIA-PLA2 was expressed and purified as described previously (24). Purified recombinant human gV and gX PLA2 were provided by Dr. M. Gelb (University of Washington, Seattle, WA) (13).

Human neutrophils

Human neutrophils from normal healthy human donors were purified from peripheral blood as described previously (30) after obtaining informed consent in accordance with a protocol approved by the institutional review board for human subjects at University of Iowa. The recovered granulocytes were resuspended in HBSS1, counted, and further diluted so that cell density was no greater than 30 × 106 PMN/ml. Cell purity was determined by microscopy after staining cells with HEMA 3 and was typically >95% neutrophils. The neutrophils were stored at room temperature for up to 1 h until use. Ten minutes before addition of neutrophils to bacterial suspensions, purified neutrophils were diluted to 105 PMN/ml in 20 mM HEPES-buffered HBSS supplemented with 1% HSA and 10% pooled human serum. These cells were then preincubated at 37°C for 10 min before addition to the bacterial suspension.

Pooled human serum

Serum was isolated from at least nine different healthy donors, pooled, filter-sterilized, aliquoted, and frozen at −80°C until use. Stored aliquots of serum were subjected to only one freeze/thaw cycle and used the day of thawing.

Opsonization of bacteria and incubation with PMN

Subcultured bacteria (105 CFU/ml) were incubated for 20 min at 37°C in 20 mM HEPES-buffered HBSS1 supplemented with 1% HSA and 10% pooled human serum. After this incubation, bacteria were mixed 1/1 (v/v) with prewarmed neutrophils to yield final cell concentrations of 5–106 PMN/ml and 5 × 106 CFU/ml. Incubations were conducted in 5–ml round-bottom polystyrene tubes swirling at 180 rpm in a water bath at 37°C for up to 5 h.

Assays of bacterial uptake by neutrophils

After incubation at 37°C, aliquots of cell suspensions containing 7.4 × 104 neutrophils were spun using a cytocentrifuge (Cytospin 2; Shandon Southern Products) at 18 × g for 10 min to sediment the cells onto poly-l-lysine-coated slides that had been primed with HEPES-buffered HBSS1 supplemented with 0.2% HSA. Cell smears were fixed and stained using the HEMA 3 stain kit according to the manufacturer’s protocol, then rinsed gently with deionized H2O. The smears were allowed to dry at room temperature and were subsequently covered with mounting medium (polyvinyl alcohol, Tris-base, glycerol, and 1,4-diazabicyclo-[2.2.2]octane; Sigma-Aldrich) and a coverslip. The number of bacterial cocci per PMN was enumerated by visualization of the samples with a Zeiss Axioplan2 photomicroscope. At least 100 neutrophils were examined per sample.

Assay of degradation of [14C]-labeled bacterial PL by purified PLA2

Subcultured [14C]oleate-labeled S. aureus (105 CFU/ml) were incubated for 20 min at 37°C in 20 mM HEPES-buffered HBSS1 supplemented with 1% HSA and 10% pooled human serum, and purified gV, gX, or gIIA PLA2 in concentration ranging from 0–1000 ng/ml. After this incubation, bacteria were mixed 1/1 (v/v) with a buffer solution containing 20 mM HEPES-buffered HBSS1 supplemented with 1% HSA and 10% pooled human serum to yield final cell concentrations of 5 × 106 CFU/ml. After incubation at 37°C for 1 h, the cell suspensions were mixed with HSA to bring the final concentration of HSA to 2%. The samples were vortexed for ~5 s, then spun for 5 min at ~10,000 × g to separate undigested [14C]PL that cosedimented with the bacteria from [14C]PL breakdown products ([14C]free fatty acids and [14C]lysyl-PL), which were quantitatively recovered as complexes with albumin (31). Aliquots of the whole suspension, before sedimentation of bacteria and the recovered supernatant were analyzed by liquid scintillation spectroscopy to determine the percentage of [14C]lipid released by the bacteria, i.e., as a quanititation of degraded [14C]labeled bacterial PL (18, 31).


After incubation of [14C]oleate-labeled S. aureus with neutrophils, cell suspensions containing [14C]-labeled S. aureus and neutrophils were extracted via a modified Bligh-Dyer method (13, 32). Briefly, whole cell suspensions (1 vol) were mixed with 6 vol of chloroform-methanol (1/2; v/v) and stored at 4°C until analyzed. Samples were spun at 1900 × g for 10 min, and the fluid was removed and transferred to a fresh glass tube, leaving the pellet behind. Three volumes of 50 mM potassium chloride and 2 vol of chloroform were vortexted and the samples were vortexed. The samples were spun for 10 min at 1900 × g to separate the water-methanol phase from the chloroform phase (bottom phase). The chloroform phase was removed and saved. The remaining aqueous phase was mixed with 4
vol of chloroform, vortexed, and spun for 10 min at 1900 x g. The chloroform phase was again removed and combined with the previously removed chloroform phase. The combined chloroform phases were dried under a stream of nitrogen, resuspended in 10 ml of chloroform/methanol (2/1, v/v), and applied to a high performance TLC plate. Lipids were resolved using a solvent system composed of chloroform, methanol, water, and acetic acid (65/25/4/1, v/v/v/v). Resolved 14C-labeled lipids were visualized by exposure of TLC plates to tritium storage phosphor screens analyzed by a Typhoon 9410 variable mode imager (Amersham Biosciences). Quantification was performed using ImageQuant software (Molecular Dynamics).

Assay of bacterial viability

After incubation at 37°C, aliquots (10 ml) of the cell suspension were added to 490 ml of 1% saponin in H2O. Samples were vortexed vigorously, incubated for 15 min at room temperature to lyse the neutrophils, and then serially diluted in HBSS. Aliquots (10–20 ml) representing 200 CFU were added to a 50-mm petri dish, followed by 5 ml of molten agar (50°C), and gently mixed. The agar was then allowed to solidify, and CFU were counted after overnight incubation at 37°C.

Assay of bacterial lysis

Subcultured bacteria (10⁸ CFU/ml) were incubated at 37°C in 20 mM HEPES-buffered HBSS supplemented with 1% HSA, 10% pooled human serum, and 1000 ng/ml recombinant gIIA PLA2. At various times, A₅₅₀ of the bacterial suspension was measured using a Bio-Rad SmartSpec3000 to monitor bacterial lysis, (i.e., reduced A₅₅₀).

Results

Comparison of serum requirements for uptake of wt and dltA S. aureus by human neutrophils

To facilitate comparison of bacterial PL degradation and, by implication, intracellular gV/X PLA2 action against ingested wt and the bacterial suspension was measured using a Bio-Rad SmartSpec3000 to monitor bacterial lysis, (i.e., reduced A₅₅₀).

FIGURE 1. Serum requirements for uptake of wt or dltA S. aureus by human neutrophils. S. aureus (10⁷ CFU/ml) were preincubated with or without the indicated amount of pooled human serum and then mixed (1/1, v/v) with human neutrophils (10⁷ cells/ml) in HBSS supplemented with 20 mM HEPES, pooled human serum, and 1% HSA (incubation medium). At the times indicated, uptake of S. aureus by neutrophils was measured as described in Materials and Methods. The results represent the mean of three experiments ± SE.

FIGURE 2. Uptake and killing of wt or dltA S. aureus by human neutrophils are not accompanied by increased bacterial PL degradation. S. aureus (10⁷ CFU/ml) labeled with [14C]oleic acid were preincubated with 10% pooled human serum in incubation medium, then mixed (1/1, v/v) with or without human neutrophils to give final cell concentrations of 5 x 10⁶ CFU/ml S. aureus and 5 x 10⁶ neutrophils/ml. At the times indicated, aliquots of the cell suspensions were assayed for bacterial uptake (A), viability (B), and PL degradation (C and D) as described in Materials and Methods. Approximately the same amount of [14C]-labeled lipids (cpm) was recovered from each sample and applied for TLC analysis. The percentages of total [14C]-labeled lipids that corresponded to PG and CL (E, wt; F, dltA S. aureus) were determined as described in Materials and Methods. The TLC panels shown (C and D) are representative of six or more experiments. The results shown in A, B, E, and F represent the mean ± SE of six or more experiments. The small difference in the number of cocci per PMN of wt vs dltA cocci associated per neutrophil corresponds to the small difference in the number of cocci per CFU in these two strains. There were no visible extracellular wt or dltA bacteria after ≥10-min incubation with neutrophils.
**dltA S. aureus**, we chose experimental conditions that permitted measurement of bacterial uptake, killing, and digestion in the same samples. After preincubation with serum to opsonize the bacteria, we incubated equal numbers of bacterial CFU and neutrophils (5 × 10⁷/ml, each). Because d-alanylation of (lipo)teichoic acids might affect the efficiency and/or consequences of important interactions of serum components with the bacterial envelope, we first compared the dose requirements for serum-dependent opsono-phagocytosis by human neutrophils (Fig. 1). Optimal uptake of wt or dltA S. aureus required ≥4% pooled human serum; at serum concentrations ≥4%, uptake of nearly all bacteria from each strain occurred within 30 min. At lower serum concentrations, bacterial uptake at 30 min was less than that at >4% serum, but increased progressively over time to levels greater than those in the initial inoculum, reflecting the gradual uptake of some of the remaining extracellular bacteria that had multiplied. In the absence of neutrophils, both strains grew exponentially in the presence of the absence of serum (Fig. 2B; data not shown). Heat inactivation of serum greatly reduced bacterial uptake, consistent with an essential role of complement in normal serum-dependent uptake of S. aureus by human neutrophils (data not shown) (33, 34). Taken together, these data demonstrate that the serum requirements were essentially the same for opsono-phagocytosis of wt or dltA S. aureus by human neutrophils.

**Effect of d-alanylation of (lipo)teichoic acids on sensitivity of S. aureus to PL degradation by human neutrophils**

To facilitate assay of bacterial PL degradation during and after phagocytosis, the membrane PL of wt or dltA S. aureus were metabolically labeled with [14C]oleic acid during bacterial growth. There was rapid and nearly complete uptake (Fig. 2B) and ≥1 log killing (Fig. 2A) of both bacterial strains. Each strain grew exponentially in the presence of serum but the absence of neutrophils (Fig. 2A). The nearly constant number of coccii (either wt or dltA) observed associated with neutrophils after 10 min to 5 h of incubation indicated rapid bacterial uptake and little or no subsequent bacterial lysis (Fig. 2B). Both strains showed a neutrophil-induced conversion of much of the major bacterial PL, PG, to CL (Fig. 2, C and D). However, even after 4-h incubation with neutrophils, there was little or no neutrophil-induced PL degradation (Fig. 2, C–F). Comparison of the microscopic appearance of the ingested bacteria after 10 min (Fig. 3, A and B) and 5 h (Fig. 3, C and D) incubation revealed modest changes in the staining properties of both wt and dltA S. aureus (see arrowheads, Fig. 3, C and D), but retention of their overall coccoid appearance. Thus, even in the absence of d-alanylation of (lipo)teichoic acids, ingested S. aureus resisted degradation of membrane PL during phagocytosis by human neutrophils and apparently retained the overall structural integrity of their envelope.

**Comparison of the sensitivities of wt and dltA S. aureus to human gIIA, V, and X PLA₂**

The absence of neutrophil-induced bacterial PL degradation during phagocytosis could reflect limiting amounts of granule-derived gV and X PLₐ₂ and/or low intrinsic antistaphylococcal activity of these granule-associated enzymes that precludes their action during phagocytosis (13, 19, 21). To test the latter possibility, we examined the sensitivity of wt and dltA S. aureus to purified gV and X PLₐ₂ as well as to gIIA PLₐ₂ for comparison. The much greater sensitivity to gIIA PLₐ₂ of the dltA strain compared with that of wt S. aureus was confirmed (Fig. 4). In contrast, neither gV nor gX PLₐ₂ was active against the wt or dltA strain, even when added at concentrations 100- to 1000-fold greater than that needed for gIIA PLₐ₂ action against the dltA strain (Fig. 4B). These findings suggest that the low intrinsic antistaphylococcal activity of gV
and gX PLA₂ precludes their contribution to neutrophil cytotoxicity against ingested *S. aureus*.

**Effect of combined presence of human gIIA PLA₂ and neutrophils on fate of wt or dltA S. aureus**

Neutrophil-rich, acute inflammatory exudates contain extracellular gIIA PLA₂ (17) (N. S. Liang, J. Lee, and J. Weiss, unpublished observation), thus exposing invading bacteria to both neutrophils and gIIA PLA₂ at the same time. Therefore, we examined the fate of wt or dltA S. aureus exposed simultaneously to neutrophils and gIIA PLA₂ as well as to serum factors needed for opsonization. GIIA PLA₂ at low concentrations (100 ng/ml; Fig. 5) resulted in dramatically greater cytotoxicity against the mutant vs the parent strain, as manifested by degradation of phospholipids, loss of CFU, and apparent lysis of the bacteria (Fig. 5). At these enzyme concentrations, gIIA PLA₂-dependent bacterial phospholipid degradation and killing of the dltA strain occurred with or without neutrophils present (Fig. 5, D and E). However, phagocytosis was nearly complete within 10 min (Fig. 2A) and thus was much faster than the rate of extracellular PLA₂ action, which required 30–60 min (24) (data not shown). In light of these differential kinetics, the data suggest that most degradation and killing seen in the presence of neutrophils occurred after bacterial ingestion. In addition, apparent lysis of the bacteria (i.e., disappearance of bacterial cocci) occurred only when the bacteria were simultaneously exposed to neutrophils and gIIA PLA₂ (Figs. 5F and 6F) and not to either neutrophils (Figs. 3D and 5F) or PLA₂ (Figs. 5F and 6D) alone. Addition of PLA₂ after the dltA bacteria had been ingested by neutrophils had much less effect than when it was added simultaneously (Fig. 5, D–F), indicating that under these circumstances, phagocytosis by neutrophils could provide a protective haven for *S. aureus*.

**Discussion**

In the present study we have reproduced the observation that there is a marked difference in the sensitivities of wt and dltA *S. aureus* to gIIA PLA₂ (24). The sensitivity of the dltA strain is so great that even small amounts of the extracellular PLA₂ (≥10 ng/ml) are sufficient to dramatically increase the antibacterial cytotoxicity effected in human neutrophil-bacterial suspensions as judged by bacterial phospholipid degradation, CFU, and number of detectable cocci. Even without neutrophils, gIIA PLA₂ at these low doses can produce nearly complete phospholipid degradation and killing of dltA *S. aureus* (Figs. 5, D and E). However, the rapidity of bacterial ingestion by neutrophils (Fig. 2A) relative to the rate of the extracellular action of PLA₂ (18, 24, 35) makes it very likely that most of the observed cytotoxic effects occur within neutrophils, amplified by PLA₂ bound to the bacteria before phagocytosis (13, 24, 25). The virtual disappearance of visible cocci after simultaneous exposure of dltA *S. aureus* to gIIA PLA₂ and neutrophils (Figs. 5F and 6F) suggests that extensive bacterial phospholipid degradation mediated by gIIA PLA₂ potentiates bacterial lysis within neutrophils, possibly by facilitating the action of neutrophil as well as bacterial cell wall-degrading enzymes (35).

The sensitivity of wt strains of *S. aureus* to gIIA PLA₂ can be significantly enhanced by exposure of the bacteria to neutrophils and requires a functional neutrophil NADPH oxidase to permit PLA₂ action at extracellular concentrations that are otherwise limiting (Fig. 5A) (13). Synergy between neutrophils and gIIA PLA₂ was not observed with the dltA mutant (data not shown), probably because this mutant strain is already so sensitive to gIIA PLA₂. Despite the exquisite intrinsic sensitivity of dltA *S. aureus* to gIIA PLA₂, they are virtually resistant to the added extracellular enzyme once they have been ingested by neutrophils (Fig. 5, D–F). Whether this simply reflects too little added enzyme gaining access.
to the PL of the ingested bacteria and/or adaptive changes that render ingested bacteria and their PL less sensitive to gIIA PLA2 remains to be determined. Phagocytosis of wt or dltA S. aureus is accompanied by conversion of much of the bacterial PG to CL (Fig. 2, C and D), probably reflecting a bacterial stress response that could increase the resistance of the bacterial envelope to gIIA PLA2 and other related PL A2 (13, 36, 37).

In marked contrast to the sensitivity of dltA S. aureus to gIIA PL A2, the structurally related secretory PL A2 present in human neutrophil granules displayed no activity against this bacterial strain either during phagocytosis (Fig. 2, C–F) or when added as purified enzymes in cell-free assays (Fig. 4B). GIIA PL A2 represents an acute phase reactant that plays a prominent role in host defense against Gram-negative and Gram-positive bacteria (4, 6, 13, 16–19, 20, 22, 23, 38, 39). Antibacterial roles of gV and gX PL A2 are much less likely (20, 22, 23; this study). Instead, the role of these enzymes during the host response to bacteria may involve their metabolism of arachidonic acid from host cell membranes (21, 40, 41). Nevertheless, gV and gX PL A2 have similar catalytic activity as that of gIIA PL A2 when the bacterial PL are presented either as purified lipid dispersions or as part of cell wall-depleted protoplasts (22–24) (N. S. Liang and J. Weiss, unpublished observations). Thus, the relative inactivity of gV and gX PL A2 compared with gIIA PL A2 toward intact bacteria probably reflects their inability to efficiently bind to and penetrate the cell wall as needed to access PL in the cell membrane (23, 24, 35). The apparent retention of the overall structural integrity of the cell wall of the ingested bacteria during and after phagocytosis by human neutrophils in the absence of gIIA PL A2 may thus be important in maintaining bacterial resistance to neutrophil PL A2.

We cannot exclude the possibility that higher concentrations (>1 μg/ml) of gV and/or gX PL A2 would have shown some activity. This may be most likely for the gV enzyme, whose cationic properties (net charge, +6) (42) would be expected to facilitate its action against dltA S. aureus (22–24). However, no circumstances are known in which either gV or gX PL A2 accumulates to such high extracellular levels. In neutrophil-rich inflammatory exudates, the much more potent gIIA PL A2 is also the more abundant enzyme (4, 18, 19, 40). Our experiments clearly show that the levels of gV and gX PL A2 in human neutrophils are insufficient to permit these enzymes to contribute to antibacterial cytotoxicity, even of the dltA mutant.

In contrast to the remarkable differences in sensitivities of wt and dltA S. aureus to gIIA PL A2, we did not observe appreciable differences in either human serum-dependent opsonization or subsequent neutrophil-dependent cytotoxicity toward the two strains in the absence of extracellular gIIA PL A2. As expected, the serum effects were heat sensitive, implying complement-dependent opsonization, and thus indicate that there was little or no effect of β-alanylation of (lipo)teichoic acids on complement fixation on the bacterial surface or subsequent engagement of neutrophil complement receptors (33, 34). The similarity of neutrophil-dependent cytotoxicity toward wt and dltA S. aureus was apparent by three independent assays conducted in the same neutrophil-bacterial suspension: bacterial phospholipid degradation, CFU, and microscopic appearance (Figs. 2 and 3). Our findings differ from those reported previously by Collins et al. (26), who showed significantly greater serum- and human neutrophil-dependent killing of dltA than of wt S. aureus using the same strains we used in this study. Attempts to date to identify which of the many differences in experimental design could account for the different observations have been unsuccessful.

In summary, our findings strongly suggest that despite the pleiotropic effects of β-alanylation of (lipo)teichoic acids on bacterial envelope properties, the absence of β-alanylation has a much greater effect on bacterial sensitivity to gIIA PL A2 than on other neutrophil antimicrobial effector systems. Extracellular gIIA PL A2 is required for bacterial PL degradation during and after phagocytosis by human neutrophils of dltA as well as dltA+ S. aureus (Fig. 5, A and D). During local and/or systemic acute inflammation, extracellular gIIA PL A2 is mobilized in a time-dependent fashion by a variety of acute inflammatory stimuli, including, but not limited to, infection by S. aureus (4, 16, 18, 19, 40) (N. S. Liang, J. Lee, and J. Weiss, unpublished observation). Inducers of gIIA PL A2 synthesis and secretion include specific bacterial products (43, 44) and host cytokines (e.g., IL-1, IL-6, TNF-α, and IFN-γ) (40, 45) that can be induced by a variety of microbial and/or host products (39), including Gram-positive bacterial products. Extracellular gIIA PL A2 under these conditions may reach concentrations as high as 1–2 μg/ml (100–1000 times above resting levels) and are sufficient for direct extracellular antibacterial action (4, 18, 19). However, early in the acute inflammatory response, when
extracellular gIIA PLA2 concentrations may be limiting, the concerted actions of extracellular gIIA PLA2 and neutrophils (13) may be critical.

Although initial gIIA PLA2 binding to wt or delTA S. aureus is the same, the much greater sensitivity of delTA S. aureus to bound PLA2 permits initiation of bacterial PL degradation at low PLA2 concentrations and additional PLA2 binding before phagocytosis (24). The greater binding and activity of bound PLA2 toward delTA S. aureus result in much more rapid and extensive bacterial membrane PL degradation before, during, and after phagocytosis and, in parallel, diminished intracellular persistence of viable bacteria or their viable remnants (Figs. 5, D and F, and 6F). These findings strongly suggest that factors regulating the mobilization and activation of PLA2 at infected sites are important determinants of the ultimate fate of S. aureus ingested by neutrophils. The extraordinary sensitivity of delTA S. aureus to gIIA PLA2 means that effective extracellular enzyme concentrations will be achieved earlier during the inflammatory response if D-alanylation of the invading bac-

References


