Effects of *Staphylococcus aureus* Leukocidins on Inflammatory Mediator Release from Human Granulocytes

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The secretion of the Panton-Valentine leukocidin (Luk-PV) but not of another leukocidin (Luk-R) from *Staphylococcus aureus* strains is correlated with severe pyodermic infections (dermonecrosis). The effects of both Luk-PV and Luk-R in amounts of 0–5000 ng on inflammatory mediator release from human leukocytes were studied. Luk-PV but not Luk-R induced a pronounced release of the vasodilator histamine from human basophilic granulocytes (up to 55% ± 7%) and of enzymes (β-glucuronidase, up to 45% ± 10%; lysozyme, up to 35% ± 7%), chemotactic components leukotriene B_4 (42 ± 8 ng/10^7 cells) and interleukin-8 (up to 33 ± 5 ng/10^7 cells), and oxygen metabolites from human neutrophilic granulocytes. The results indicate that granulocytes play a central role in dermonecrosis; these in vitro data account for the histologic picture of Luk-PV infections, characterized by local vasodilation, infiltration of granulocytes, and a central necrotic area.

Staphylococcus aureus strains are associated with a variety of diseases such as lung infection, burn wound sepsis, furuncles, and dermonecrosis [1–3]. Among the various pathogenicity factors of *S. aureus*, toxins play an important role in disease processes [1–7]. Previous studies have shown that staphylococcal toxins (e.g., α toxin, Δ toxin, and toxic shock syndrome toxin) may induce or modulate release of inflammatory mediator from human polymorphonuclear leukocytes (PMNL) for subsequent stimulation of the target cells [1, 4–7].

Staphylococcal Panton-Valentine leukocidin (Luk-PV) and leukocidin R (Luk-R) are two-component toxins; the two components (S and F) act synergistically on leukocytes and thus belong to the new family of synergohymenotropic toxins [8–10]. Luk-PV was originally isolated from *S. aureus* V8; Luk-R was first obtained from *S. aureus* P83, isolated from an infected bovine udder [9, 11, 12]. Recently, both Luk-PV and Luk-R were obtained from *S. aureus* V8 [10]. The combination of Luk-PV S and F components is highly leukotoxic only for human and rabbit PMNL; it does not induce hemolysis [10, 12]. On the contrary, the combined S and F components of leukocidin R have hemolytic activity and are leukotoxic for PMNL of a broad range of animal species, including humans [10]. Predominantly Luk-PV–producing strains are mostly associated with infections such as furuncles and abscesses as well as with severe pyodermic infections such as dermonecrosis [10, 11, 13]. Histologically, dermonecrosis is characterized by a central necrotic area surrounded by massive infiltration by PMNL and macrophages [11].

Human granulocytes participate as effector cells of the first defense line in the regulation of normal immune functions. Human granulocytes respond to a variety of stimuli (e.g., bacteria, toxins) with the release of mediators after degranulation of preformed constituents (e.g., histamine, enzymes) or after novel generation (e.g., oxygen metabolites, leukotrienes, interleukin [IL]-8). However, an inappropriate immune response may lead to tissue injury and promotion of inflammation [14, 15].

Leukotriene B_4 (LTB_4) is a potent chemotactic factor for granulocytes [16]. It exerts autocrine effects on PMNL and induces neutrophils to degranulate, to generate superoxide, and to adhere to the vascular endothelium [15]. IL-8, a recently described 6- to 10-Kda protein, is produced by a variety of cells in vitro, including peripheral blood leukocytes, and represents a cytokine that induces chemotaxis, degranulation, respiratory burst, adherence, shape change, Ca^{2+} mobilization, and up-regulation of CD11b/CD18 glycoprotein in human PMNL [17–20]. Enzyme release (e.g., lysozyme) and the generation of oxygen metabolites are primarily directed against invading microorganisms [16]. On stimulation, basophilic granulocytes release preformed mediators such as histamine, which increases vascular permeability [16].

Our study analyzed the pathophysiologic requirements leading to cutaneous abscesses or to necrotizing cutaneous infections, including dermonecrosis, induced by *S. aureus* strains. Therefore, we studied the interaction of two *S. aureus* toxins, Luk-PV and Luk-R, with human granulocytes with regard to inflammatory mediator release (chemolumines-

Received 14 March 1994; revised 11 October 1994.

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The Journal of Infectious Diseases 1995;171:607–13

0022-1899/95/7103-0013$01.00

cence, enzymes, histamine, LTB_4, IL-8) and cytotoxicity (trypan blue exclusion).

Methods

Buffer. The buffer used for washing the cells and for mediator release consisted of 137 m*M* NaCl, 8 m*M* Na_2HPO_4, 3 m*M* KCl, and 3 m*M* KH_2PO_4, pH 7.4 (modified Dulbecco's PBS).

Cell viability. Cell viability was studied by trypan blue exclusion and by analysis of lactate dehydrogenase (LDH) release from stimulated and nonstimulated cells. Analysis of LDH (EC 1.1.1.27) was done as described previously [16].

Toxin purification. Luk-PV and Luk-R were purified from *S. aureus* V8 as described [10, 12]. In all experiments, the individual components (F and S) of Luk-PV or Luk-R or the complete toxins (F and S components combined) were used.

Preparation of polymorphonuclear neutrophilic and basophilic granulocytes. Human PMNL were isolated from 200 mL of heparinized blood (15 units/mL) from 3 healthy donors; cells were separated on a ficoll-metrizoate gradient, followed by dextran sedimentation as described previously [16, 21]. The LMB fraction containing 84.6% ± 4.6% lymphocytes (L), 14.2% ± 4.1% monocytes (M), and 1.2% ± 0.5% basophilic granulocytes (B) was isolated by ficoll-metrizoate sedimentation and washed twice with PBS at 300 *g* [21, 22]. The PMNL were isolated by dextran sedimentation and washed twice at 300 *g*. This method led to >95% pure PMNL. The cells were diluted to a final concentration of 2×10^7 cells/mL in PBS.

Cellular activation: enzyme and histamine release, leukotriene and IL-8 generation. Human PMNL (10^7/500 μL) or human LMB (5×10^6/500 μL) were stimulated in the presence of Ca^{2+}/Mg^{2+} (0.6/1 m*M*) with 50 μL of buffer (control) or with 50 μL of the appropriate stimulus for the indicated periods at 37°C. The appropriate supernatants of stimulated cells were analyzed for LTB_4, histamine, enzymes, and IL-8.

Histamine release from human basophilic granulocytes. The washed cells (5×10^6 LMB/500 μL) were stimulated in the presence of Ca^{2+} (1 m*M*) and Mg^{2+} (0.5 m*M*) with 50 μL of PBS (buffer control), with formylmethionylleucylphenylalanine (FMLP, 10^{-4} *M*), with Luk-PV (components S, F, and S plus F), or with Luk-R (S, F, and S plus F) for 60 min at 37°C. Cells were centrifuged for 15 min at 300 *g*, deproteinized by the addition of $HClO_4$ (2%, 2 mL), and subsequently analyzed for histamine contents by the fluorophotometric analyzer technique (Autoanalyzer; Technicon, Bad Vilbel, Germany) [16, 23]. Histamine dihydrochloride dissolved in 2% $HClO_4$ served as a control. Cells in the presence of buffer and the bacterial supernatant at the appropriate dilutions served as negative controls. For determination of the total cellular histamine content (100%), cells were disrupted by the addition of 2 mL of $HClO_4$.

Stimulation of PMNL and analysis of leukotrienes. PMNL (10^7/500 μL) were stimulated in the presence of Ca^{2+} (1 m*M*) and Mg^{2+} (0.5 m*M*) with 50 μL of PBS (buffer control), with FMLP (10^{-4} *M*), with the calcium ionophore A23187 (6.3 μ*M*), or with the appropriate stimulus for the indicated times at 37°C. The reaction was terminated after addition of 2 mL of methanol/acetonitrile (50/50, vol/vol). Samples were processed and analyzed as described [16]. In brief, the deproteinized samples were centrifuged (1200 *g*, 15 min), the supernatant evaporated, and the residue resuspended in 500 μL of methanol/water (30/70, vol/vol) and analyzed by high-performance liquid chromatography. The leukotrienes were identified and quantified by area integration of the absorption peaks at 280 nm and external standardization with synthetic standards.

IL-8 assay. IL-8 release was determined using a sandwich ELISA according to a published method [24]. Each well of a 96-well plate (Maxisorb; Nunc, Roskilde, Denmark) was precoated overnight at 4°C with 100 μL of PBS–Tween 20 (0.1%) containing anti–IL-8 antibodies at 5 μg/mL. The plates were washed three times with PBS-Tween, the appropriate samples (or standard recombinant human IL-8; Calbiochem, Bad Soden, Germany) were added, and incubation proceeded for 2 h at 37°C. Then alkaline phosphatase–linked anti–IL-8 antibody was added. After addition of *p*-nitrophenylphosphate (15 mg/mL), an ELISA reader was used for quantification and Mikrotek software (SLT Labinstruments, Crailsheim, Germany) was used for calculations.

Determination of enzyme release (β-glucuronidase, lysozyme, and LDH) from human PMNL. The release of β-glucuronidase, lysozyme, and LDH was determined as described [16]. Enzyme activities were calculated as the percentage of total enzyme activities available after sonication of unstimulated PMNL (10^7).

Chemoluminescence response of human granulocytes. Oxygen radical production was monitored by the luminol-enhanced chemoluminescence as described elsewhere [16]. PMNL (10^6/320 μL of PBS) in the presence of 0.6 m*M* calcium, 1 m*M* magnesium, and 0.25 m*M* luminol were incubated for ~15 min at 37°C until a stable signal was obtained. Subsequently, 50 μL of PBS (buffer control), phorbol myristate acetate (PMA, 10^{-8} *M*), FMLP (10^{-4} *M*), or the appropriate stimulus was added. The production of oxygen radicals (chemoluminescence) was monitored (Lumacounter M2080; Lumac, Schaesberg, Netherlands) over the indicated intervals.

Statistics. All data were calculated as means ± SD ($n = 3$) from 3 donors. Significance was evaluated by Student's *t* test for independent means.

Results

Effect of Luk-PV and Luk-R on histamine release from human LMB. Histamine presents a preformed mediator with potent vasodilatory effects. Histamine is released from human basophilic granulocytes and from mast cells. We studied the effects of Luk-PV and Luk-R on histamine release from human basophilic granulocytes. The experimental design was chosen from prior experiments [16]. Human LMB were incubated in the presence of Luk-PV (S plus F), Luk-R (S plus F), or the individual components at different concentrations (3000, 2000, 1000, 500, 50, 5, or 0 ng) for 1 h at 37°C; for comparison, LMB were stimulated with FMLP (10^{-4} *M*). As is apparent from figure 1, Luk-PV induced a more pronounced histamine release than did Luk-R. While 1000 ng of each component of Luk-PV induced a histamine release of 55% ± 7%, 3000 ng of each component of Luk-R

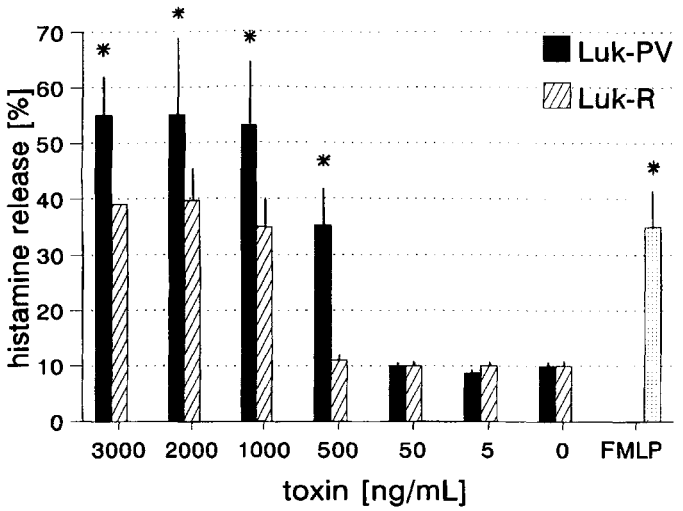

Figure 1. Effects of leukocidins Luk-PV and Luk-R on histamine release from human basophilic granulocytes. Human lymphocytes, monocytes, and basophilic granulocytes (5×10^6) were stimulated with Luk-PV or Luk-R at indicated concentrations (0, 5, 50, 500, 1000, 2000, or 3000 ng) for 1 h at 37°C. Formylmethionylleucylphenylalanine (FMLP, 10^{-4} *M*) stimulation was done as positive control. Data are mean ± SD of 3 experiments. * Significant difference vs. Luk-R.

induced a histamine release of 39% ± 5% (total histamine content = 100%). A further increase in Luk-R concentration (up to 5000 ng of each component) did not enhance histamine release. The individual components (S, F) of Luk-PV and Luk-R failed to induce histamine release from human LMB at the concentrations tested (0–3000 ng, data not shown). Thus, in comparison with FMLP (35% ± 7% histamine release), both *S. aureus* toxins (Luk-PV and to a lesser degree Luk-R) were potent inducers of histamine release from human basophilic granulocytes.

Leukotriene generation from human PMNL by Luk-PV and Luk-R. To study the effects of Luk-PV and Luk-R on the generation of chemotactically active LTB_4, human PMNL (10^7/500 μL) were stimulated with Luk-PV (S plus F), Luk-R (S plus F), or with the individual components at concentrations of 0–3000 ng for 30 and 60 min at 37°C. For comparison, PMNL were stimulated with the calcium ionophore A23187 (6.3 μ*M*) and FMLP (10^{-4} M). As shown in figure 2, Luk-PV and Luk-R induced the generation of LTB_4 during the 30 min incubation. For Luk-PV, maximal LTB_4 generation (total LTB_4 = LTB_4 + Ω products) was obtained at a Luk-PV concentration between 500 and 1000 ng of each component. A further increase of the toxin concentration up to 2000 ng did not lead to additional enhancement of LTB_4 generation; at 3000 ng of each component, LTB_4 generation from human PMNL declined. Maximal LTB_4 generation from Luk-R–treated PMNL was observed at 1000 ng, resulting in a plateau up to a Luk-R concentration of 3000 ng of each component. Nonetheless, the amounts of LTB_4 were less than those stimulated by Luk-PV. The majority of LTB_4 was detected in its Ω oxidated state. Maximal LTB_4 generation (LTB_4 + Ω products) by Luk-PV and Luk-R were obtained after 30 min at all toxin concentrations (data not shown). The S and F components of Luk-PV and of Luk-R at concentrations of 5–3000 ng failed to induce LTB_4 generation (data not shown). Luk-PV was a more potent stimulus of LTB_4 generation than was FMLP (20.1 ± 8.7 ng; figure 2) but was less active than the calcium ionophore A23187 (250 ± 100 ng; data not presented).

Effect of Luk-PV and Luk-R on IL-8 release from human PMNL. IL-8 represents another important chemotactic factor for PMNL. Therefore, we studied the effects of Luk-PV, Luk-R, and their individual components on IL-8 release from human PMNL. Human PMNL (10^7/500 μL) were stimulated with Luk-PV (S plus F), Luk-R (S plus F), or the individual components at various concentrations (2000, 1000, 500, 50, or 5 ng) for up to 60 min at 37°C. PMNL treated with Luk-PV induced a more pronounced IL-8 release than did Luk-R (figure 3). Maximal IL-8 release was observed at a Luk-PV or Luk-R concentration of 500 ng; at higher concentrations, the Luk-PV–induced IL-8 release declined. For Luk-R, a plateau of IL-8 release was reached at 500 ng.

Chemoluminescence response. We studied the effects of Luk-PV and Luk-R on the chemoluminescence response from human PMNL. Human PMNL were stimulated with

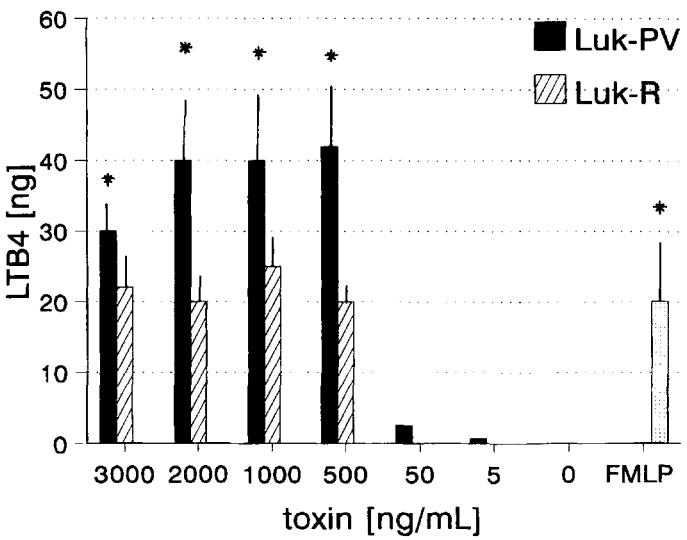

Figure 2. Effects of leukocidins Luk-PV and Luk-R on leukotriene B_4 generation from human polymorphonuclear leukocytes (PMNL). Human PMNL (10^7/500 μL) were stimulated with Luk-PV or Luk-R at indicated concentrations (0, 5, 50, 500, 1000, 2000, or 3000 ng) for 30 min at 37°C. Formylmethionylleucylphenylalanine (FMLP, 10^{-4} *M*) stimulation was done as positive control. Data are total leukotriene B_4 (LTB_4 + 20-COOH-LTB_4 + OH-LTB_4) from human PMNL, mean ± SD of 3 experiments. * Significant difference vs. Luk-R.

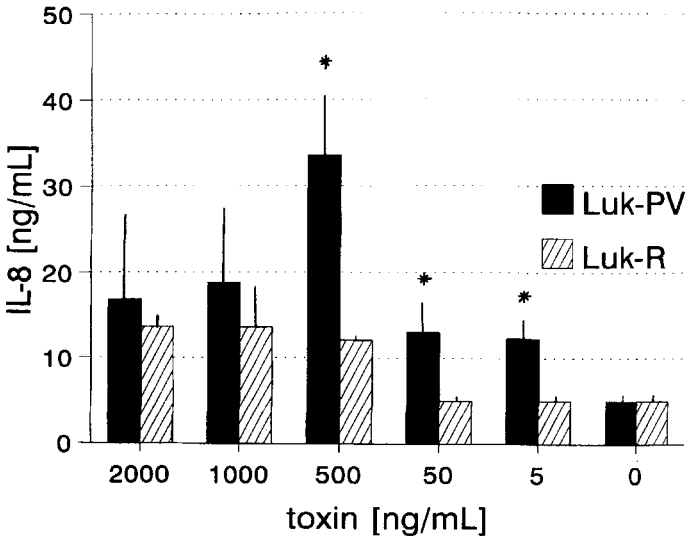

Figure 3. EffeMt of leukocidins Luk-PV and Luk-R on interleukin (IL)-8 release from polymorphonuclear leukocytes (PMNL). Human PMNL (10^7) were stimulated with Luk-PV or Luk-R at indicated concentrations (0, 5, 50, 500, 1000, or 2000 ng) for 60 min at 37°C. Data are mean ± SD (n = 8). * Significant difference vs. Luk-R, $P < .05$.

Luk-PV (S plus F), Luk-R (S plus F) or the individual components at different concentrations (1000, 500, 50, 5, or 0 ng) for up to 20 min at 37°C. For comparison, PMNL were stimulated with PMA (10^{-8} M) or FMLP (10^{-4} M) for up to 20 min at 37°C. Luk-PV (figure 4A) and Luk-R (figure 4B) differed markedly in the profile of induced chemoluminescent response. Maximal chemoluminescence was obtained at 50 ng of each component of Luk-PV; at higher Luk-PV concentrations (500 or 1000 ng of each component), the chemoluminescence declined below baseline levels.

Luk-PV (50 ng)–induced chemoluminescence was less pronounced than that produced by PMA (maximum: 500×10^3 cpm) but was similar to that induced by FMLP (maximum: 80×10^3 cpm; data not shown). In contrast to Luk-PV, Luk-R induced a maximal chemoluminescence response at concentrations of 500–1000 ng; at higher Luk-R concentrations, the chemoluminescence declined to baseline levels. Nonetheless, the chemoluminescent response by Luk-R was less pronounced than that induced by Luk-PV. The S and F components by themselves at concentrations of 5–1000 ng showed no effects on the chemoluminescence from human PMNL (data not shown).

Enzyme release (β-glucuronidase, lysozyme, LDH) from human PMNL. To study enzyme release from toxin-treated cells, PMNL (10^7/500 μL) were stimulated with Luk-PV (both components), Luk-R (both components), or the individual components (S, F) at different concentrations (3000, 2000, 1000, 500, 50, 5, or 0 ng) for up to 120 min at 37°C. For comparison, the calcium ionophore A23187 (3 μM) was used as stimulus. For an incubation time of 30 min at 37°C, Luk-PV and Luk-R showed different activation patterns for β-glucuronidase (figure 5A) and lysozyme release (figure 5B) from human PMNL. Luk-PV induced a pronounced enzyme release of up to 35% ± 7% (lysozyme) and 45% ± 10% (β-glucuronidase); Luk-R was less effective (15% ± 8% and 20% ± 5%, respectively). The S and F components individually failed to induce enzyme release from human PMNL. Thus, Luk-PV was as active as the calcium ionophore A23187 (β-glucuronidase, 32% ± 7%; lysozyme, 25% ± 4%) on enzyme release from human PMNL. A significant increase in

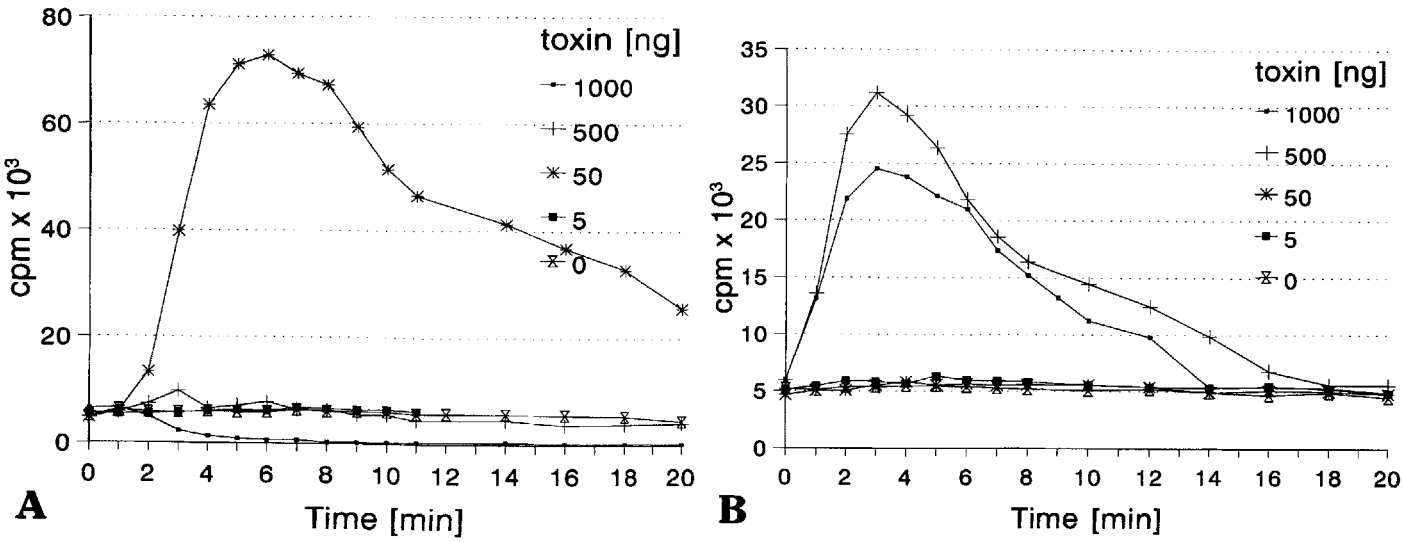

Figure 4. Chemoluminescence response from human polymorphonuclear leukocytes (PMNL). Human PMNL were stimulated with leukocidin Luk-PV (**A**) or Luk-R (**B**) at indicated concentrations (0, 5, 50, 500, or 1000 ng) for up to 20 min at 37°C. Data are means of 5 experiments.

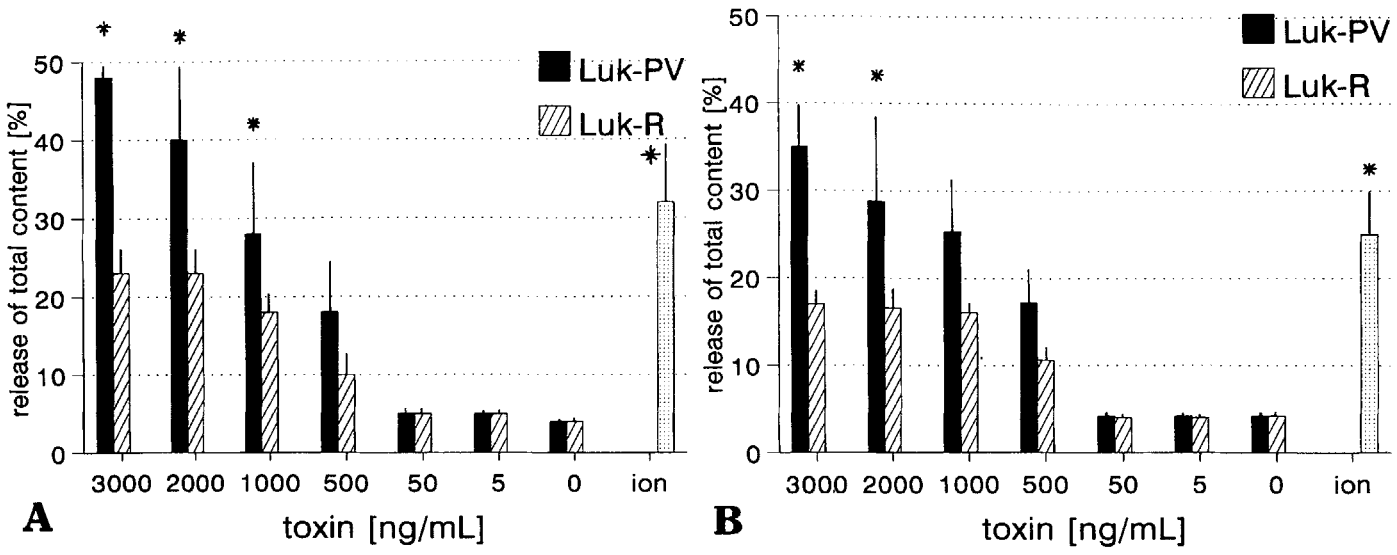

Figure 5. Release of preformed mediators β-glucuronidase (**A**) and lysozyme (**B**) from human polymorphonuclear leukocytes (PMNL). Human PMNL (10^6/500 μL) were incubated with leukocidin Luk-PV or Luk-R at indicated concentrations (0, 5, 50, 500, 1000, 2000, or 3000 ng) for 60 min at 37°C. Calcium ionophore A23187 (ion; 3 μ*M*) stimulation was done as positive control. Data are mean ± SD of 7 experiments. * Significant difference vs. Luk-R, $P < .05$.

LDH release compared with buffer control was not detected independent of the toxin used up to an incubation time of 120 min (data not shown).

Effects of Luk-PV and Luk-R on trypan blue staining of human PMNL. Trypan blue staining was investigated as a parameter of membrane and cellular integrity. Human PMNL (10^7/500 μL) were incubated with Luk-PV (both components), Luk-R (both components), or the individual components (S, F) of both leukocidins at different concentrations (3000, 2000, 1000, 500, 50, 5, or 0 ng) for up to 3 h at 37°C. Treatment of PMNL with Luk-PV led to a dose-dependent trypan blue staining of cells. At 500, 50, or 5 ng of Luk-PV/10^7 cells, trypan blue stained 86% ± 7.2%, 19.2% ± 4.9%, and 6.0% ± 2% of PMNL (buffer control, 5.0% ± 1%). With Luk-R as stimulus, trypan blue staining >5% was not observed up to a tested concentration of 3000 ng (of each component).

Discussion

S. aureus strains are strongly associated with human cutaneous infections, including dermonecrosis [11, 13]. A prospective epidemiologic study done with 332 *S. aureus* clinical isolates established that Luk-PV–producing strains were mostly associated with necrotizing cutaneous infections. In this regard, 86% of furuncles, 37% of primitive cutaneous abscesses, and 28% of whitlows were due to Luk-PV–producing strains [10, 13]. The results of our study present new insights into the pathophysiology of skin infection caused by *S. aureus*.

Histologically, furuncles and dermonecrosis are characterized by an infiltration of PMNL and macrophages into the tissue and by necrotic areas. Cell infiltration into tissue may be associated with vasodilation (e.g., by histamine). Our data clearly show that Luk-PV is a potent inducer for histamine release from human basophilic granulocytes as well as from mast cells (rat mast cells; data not shown). Thus, Luk-PV favors local vasodilation and, in consequence, infiltration of inflammatory cells. IL-8 presents a potent inflammatory mediator with chemotactic properties for neutrophils and is produced from a variety of cells, such as monocytes, endothelial cells, and granulocytes [25]. In our study, Luk-PV clearly was a potent inducer of IL-8 release from human PMNL. Since IL-8 is regarded as a neutrophil chemotactic factor, it may be suggested that once granulocytes are present at the site of inflammation, the continuous generation of IL-8 leads to a perpetuation of neutrophil influx [17].

Although crucial in defense against infection, neutrophils and their toxic contents and products have been implicated in the pathogenesis of tissue injury in important inflammatory diseases [26]. In this regard, the neutrophil generates toxic oxygen metabolites and contains powerful degradative granule enzymes and toxic cationic proteins. Luk-PV is a potent inducer of enzyme release from granulocytes, as was shown by the detection of lysozyme and β-glucuronidase release. In addition, Luk-PV initiated a potent chemoluminescence response within a narrow concentration range.

LTB_4 belongs to the group of newly generated mediators from stimulated human PMNL. LTB_4 exerts autocrine effects on PMNL and may induce neutrophils to degranulate, generate superoxide, and adhere to the vascular endothelium. Amounts of 500–1000 ng of toxin per 10^7 cells can generate in 30 min a maximal production of LTB_4 from hu-

man PMNL [27]. Thus, the continuous generation of superoxide and release of degradative enzymes may favor tissue destruction.

Recently, Luk-PV–producing strains have been reported to simultaneously express the leukocidin Luk-R [11]. Our data indicate that *S. aureus* toxins Luk-PV and Luk-R differ markedly in their interaction with human granulocytes. Luk-R appears to be less toxic; it induced the release of histamine or enzymes or the generation of oxygen metabolites, LTB_4, and IL-8 to a lesser degree than did Luk-PV. In contrast to Luk-R, only Luk-PV caused loss of membrane integrity as measured by trypan blue exclusion. Whether Luk-PV triggers necrosis or apoptosis in human PMNL is not yet clear [28]. However, both events impair the functional activity necessary for an appropriate host defense. Should neutrophils die by necrosis and disintegrate in situ at inflamed sites, the release of their contents may exacerbate local tissue injury.

In our study, the in vitro data account for the in vivo results and the histologic picture of Luk-PV infection. Furthermore, our results are supported by an animal model. In a rabbit experimental model, intradermal injection (300 ng) of both purified components of Luk-PV resulted in an important local inflammation with a zone of central necrosis. On the other hand, injection of 30,000 ng of both purified components of Luk-R induced inflammation without necrosis [10, 11]. Histologically, all these injections led to local vasodilation and infiltration by PMNL and macrophages in the surrounding tissues, but only Luk-PV led to perivascular lysis of PMNL and necrosis. The central role of PMNL and of molecules released by PMNL for Luk-PV–induced dermonecrosis is strengthened by the observation that animals whose PMNL are insensitive to the toxin (e.g., sheep, mouse, rat, guinea pig) never develop necrosis when intradermally injected with Luk-PV, whereas those whose PMNL are sensitive (e.g., human, rabbit) develop necrotic cutaneous lesions in the presence of Luk-PV [10].

Staphylococcal Luk-PV and Luk-R protein components S and F act synergistically to induce cytotoxic changes in human and rabbit PMNL and macrophages [10]. These components, when tested individually, are noncytotoxic. Inflammatory mediator release was apparent only when human PMNL were treated with both components simultaneously or in the following sequence: LukS-PV, washing, LukF-PV. The opposite sequence (i.e., LukF-PV, washing, LukS-PV) did not evoke any biologic activity (data not shown).

Until now, the mechanism of action of Luk-PV and Luk-R was not clear. Prediction of secondary structures (DNAStar software; DNAStar, London) revealed that the nucleotide sequences of the genes from Luk-PV and Luk-R share a homology of 74%. In this regard, LukS-PV was similar to LukS-R; nevertheless, the predicted secondary structure of LukF-PV was different from those of the other F-related components [10]. Cell binding measurements with iodinated proteins indicated that binding of Luk-PV seemed to allow the secondary interaction of Luk-PV with the cell membrane, leading to calcium influx [29]. This proposed mechanism is characteristic for a pore-forming toxin. The degradation of the cell membrane is associated with stimulation of phosphatidylinositol metabolism and inhibition of cAMP-dependent protein kinase [30–33].

It is widely accepted that inflammatory disease may result from an imbalance between the load of toxic agents and tissue defense mechanisms. Thus, we conclude that the ability of Luk-PV to stimulate and to destroy PMNL may account in part for its mode of action.

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