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RESEARCH NOTE

Panton–Valentine leukocidin is expressed at toxic levels in human skin abscesses

C. Badiou[1], O. Dumitrescu[1], M. Croze[1], Y. Gillet[1,2], B. Dohin[3], D. H. Slayman[4], B. Allaouchiche[4], J. Etienne[1], F. Vandenesch[1] and G. Lina[1]

[1]INSERM U851, Lyon, Université de Lyon, Centre National de référence des Staphylocoques, Faculté Laennec, [2]Service de Réanimation Pédiatrique, Hôpital Edouard Herriot, Hospices Civils de Lyon, [3]Services de Chirurgie Pédiatrique, Hôpital Edouard Herriot, Hospices Civils de Lyon and [4]Département d'Anesthésie, Hôpital Edouard Herriot, Hospices Civils de Lyon, Lyon, France

ABSTRACT

Pus samples were prospectively collected from patients with *Staphylococcus aureus* skin infections and tested for Panton–Valentine leukocidin (PVL). PVL was detected at concentrations that were toxic for rabbit skin in all specimens from patients infected with strains harbouring PVL genes.

Staphylococcus aureus is an important human pathogen that expresses a variety of exoproteins, including Panton–Valentine leukocidin (PVL) [1]. PVL genes are carried by community-acquired methicillin-resistant *S. aureus* (CA-MRSA) clones that are spreading throughout the world [2,3].

Corresponding author and reprint requests: G. Lina, Centre National de Référence des Staphylocoques, INSERM U851, 7 rue Guillaume Paradin, 69372 Lyon cedex 08, France
E-mail: gerard.lina@chu-lyon.fr

Table 1. Panton–Valentine leukocidin (PVL) concentrations in human skin abscesses

Staphylococcus aureus isolates			Skin abscesses					
Number	*luk-PV* detection[a]	*mecA* gene detection[a]	PVL quantification by ELISA (mg/L), mean ± SD	Diameter (mm)	Subcutaneous extension	Fever, dizziness	Recurrent abscess	Antibiotic before drainage
7 304 284	+	−	0.27 ± 0.05	26	Yes	Yes	No	Amoxiclav[b]
7 666 159	+	−	0.41 ± 0.03	20	Yes	No	Yes	No
7 182 102	+	−	0.42 ± 0.01	40	Yes	Yes	No	Amoxiclav
7 203 017	+	−	0.92 ± 0.01	NR	Yes	No	Yes	Pristinamycin
6 022 246	+	−	0.99 ± 0.01	40	Yes	Yes	No	No
6 154 246	+	−	1.17 ± 0.08	50	Yes	Yes	Yes	Amoxiclav
7 161 319	+	−	1.19 ± 0.17	30	No	No	No	No
6 101 308	+	−	1.34 ± 0.07	NR	Yes	No	Yes	No
7 162 452	+	+	1.64 ± 0.08	51	Yes	Yes	No	Amoxiclav
7 283 336	+	−	1.72 ± 0.28	10	Yes	No	No	No
3 374 251	+	−	1.82 ± 0.08	35	No	NR	No	No
6 216 012	+	−	>1[c]	NR	Yes	NR	Yes	No
7 341 038	+	−	>1[c]	NR	NR	NR	NR	NR
7 343 232	+	−	>1[c]	NR	NR	NR	NR	NR
7 233 478	+	−	>1[c]	51	Yes	Yes	No	No

NR, not recorded.
[a]The *luk-PV* and *mecA* genes were detected by PCR as previously described [3].
[b]Amoxicillin–clavulanic acid.
[c]Owing to the small available volume of pus, the precise concentration of PVL could not be established.

PVL is a bi-component pore-forming exotoxin that targets cells of the immune system, e.g. polymorphonuclear neutrophils, monocytes and macrophages [4]. *In vitro*, PVL kills human and rabbit polymorphonuclear neutrophils through necrosis, whereas intradermal PVL injection in rabbits provokes skin erythema followed by dose-dependent necrosis [4,5].

PVL production has been epidemiologically linked to specific human *S. aureus* infections, such as primary skin and soft tissue disease, severe necrotizing pneumonia, and severe bone and joint infections [6–8], regardless of methicillin sensitivity. This suggests that PVL may play a role in infections due to *S. aureus* strains harbouring PVL genes, but there is still no direct evidence that PVL is produced at toxic concentrations *in vivo*.

The aim of this study was to determine whether PVL is produced in human *S. aureus* abscesses and, if so, whether it reaches potentially toxic concentrations. Pus samples (≥300 μL) were prospectively collected from patients with furuncles or abscesses (cutaneous or subcutaneous) during a 6-month period at Edouard Herriot Hospital, Lyon, France. Pus was tested for *S. aureus*, and aliquots were stored at –20°C for PVL quantification. *S. aureus* was identified on the basis of colony and cell morphology, coagulase testing with rabbit plasma (bioMérieux, Marcy l'Etoile, France), and the Staphyslide agglutination test (bioMérieux). Genomic DNA was extracted for PCR analysis with a standard procedure, and its concentration was estimated spectrophotometrically. Sequences specific for the PVL genes (*lukS-PV* and *lukF-PV*, forming the *luk-PV* operon) and the *mecA* gene (coding for methicillin resistance) were detected by PCR, and multilocus sequence typing of *mecA*-positive and *luk-PV*-positive isolates was performed as previously described [3]. In parallel, pus samples positive for *S. aureus* (regardless of *luk-PV* and *mecA* status) were blindly analysed in duplicate with a solid-phase sandwich ELISA, using a mouse monoclonal antibody and a rabbit peroxidase-conjugated polyclonal F(ab)'2 fragment targeting LukS-PV, as recommended by the supplier (R&D Immunoassays, BioMérieux, Marcy l'Etoile, France). Briefly, samples were thawed to +4°C, vortexed for 1 min, sterilized by 1 h of heating at 94°C, followed by cooling at +4°C on ice for 5 min, and centrifuged at 10 000 *g* for 5 min. PVL quantification was performed in the supernatant in duplicate. The ELISA detection limit of PVL was 50 ng/mL.

Pus from 41 patients was studied, and all samples yielded *S. aureus*. Fifteen isolates were positive for *luk-PV* according to PCR. One of these 15 isolates, of sequence type (ST) 80, was also *mecA*-positive and belonged to the European CA-MRSA clone. PVL was detected with ELISA in all pus samples that grew *luk-PV*-positive isolates. The PVL concentration ranged from 0.27 mg/L to over 2 mg/L, and exceeded 1 mg/L in the pus that yielded the *mecA*-positive isolate (Table 1). PVL was not detected by ELISA in any of the 26 samples from *luk-PV*-negative cases, including

the two *mecA*-positive cases. In patients with skin abscesses due to PVL-producing strains, a correlation between abscess diameter and the occurrence of fever and dizziness was observed (Mann–Whitney test, p 0.037). Abscess diameter and PVL concentration were not statistically correlated, due to the small population and to missing information; however, only abscesses with a PVL concentration above 1 mg/L were associated with large abscesses (diameter ≥5 cm), suggesting a possible dose effect of PVL on severity. No statistically significant correlation was found between the level of PVL and subcutaneous extension, recurrence, and the antibiotic used before drainage.

These results clearly show that PVL is expressed in human skin abscesses due to *S. aureus* strains harbouring *luk-PV* genes. Susceptibility to PVL is species-dependent. Intradermal injection of 30 ng of PVL induces oedema and erythema in rabbits, and injection of 300 ng provokes widespread and infiltrated erythema followed by skin necrosis. In contrast, doses of up to 3 μg cause no skin lesions in mice [5]. There are no corresponding data concerning humans, but it is noteworthy that human and rabbit leukocytes are sensitive to similar concentrations of PVL [9], indirectly suggesting that the levels observed here are probably toxic for human skin.

Deleo *et al.* observed no differences in the pathogenicity of PVL-positive and PVL-negative isogenic strains of *S. aureus* in a mouse model of skin abscess [10], possibly owing to the above-mentioned relative insensitivity of mouse skin to PVL at the concentrations reached *in vivo*.

The pus sample from the patient infected with the most prevalent European clone of CA-MRSA (the ST80 clone) contained a relatively high PVL concentration. It was recently found that all *luk-PV*-positive CA-MRSA strains actually produce PVL [11], and that the clinical manifestations of PVL-positive *S. aureus* infections are unrelated to methicillin susceptibility [12]. Thus, the apparent role of PVL in the pathophysiology of human skin infections involves not only PVL-producing MSSA, but also CA-MRSA.

This must be confirmed, however, by studies in areas with a high incidence of PVL-positive CA-MRSA infections. The *in vivo* PVL production by strains causing other infections, e.g. severe necrotizing pneumonia [7] and severe bone and joint infections [8], as well as the possible correlation between PVL level and severity of disease, should also be investigated.

Finally, these results further support the inclusion of PVL in the list of *S. aureus* virulence factors involved in the pathophysiology of human skin infections.

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TRANSPARENCY DECLARATION

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RESEARCH NOTE

Seasonal changes in climatic parameters and their relationship with the incidence of pneumococcal bacteraemia in Denmark

T. Tvedebrink[1,2], S. Lundbye-Christensen[1,3], R. W. Thomsen[4], C. Dethlefsen[2] and H. C. Schønheyder[5]

[1]Department of Mathematical Sciences, Aalborg University, [2]Center for Cardiovascular Research, Aalborg Hospital, Aarhus University Hospital, [3]Department of Health Science and Technology, Aalborg University, [4]Department of Clinical Epidemiology, Aarhus University Hospital and [5]Department of Clinical Microbiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

ABSTRACT

The seasonal variation in the incidence of invasive pneumococcal disease is well recognized, but little is known about its relationship with actual changes in climatic parameters. In this 8-year longitudinal population-based study in Denmark, a harmonic sinusoidal regression model was used to examine whether preceding changes in climatic parameters corresponded with subsequent variations in the incidence of pneumococcal bacteraemia, independently of seasonal variation. The study shows that changes in temperature can be used to closely predict peaks in the incidence of pneumococcal bacteraemia with a time-lag of 16 days (95% CI 14–18 days), independently of a strong seasonal pattern.

The seasonal variation in the incidence of invasive pneumococcal disease, with a peak in incidence during the winter months, is well recognized [1–4]. However, few detailed studies of the temporal relationship between actual climatic changes and subsequent pneumococcal disease are available. An 8-year longitudinal population-based ecological study was undertaken in a Danish county to examine whether changes in climatic parameters could be used to predict variations in the incidence of pneumococcal bacteraemia (PB).

Included were cases of PB that occurred from January 1995 through December 2002 in North Jutland County, Denmark, with an average population of 492 845 during the period of study, corresponding to 9% of the total Danish population. Patients with PB were identified in the County Bacteraemia Registry, which comprises blood culture results for the entire county [5]. PB was defined as an episode of clinical disease, with *Streptococcus pneumoniae* detected by blood culture [5].

Meteorological data, i.e. daily summaries from a weather station corresponding to the area under study, were obtained from the Danish Institute of Agricultural Sciences. Daily mean values were calculated for six pre-specified meteorological variables: temperature (minimum, mean and maximum), relative humidity, precipitation and wind velocity. Monthly means and sums of these variables were used in the data analysis.

A harmonic sinusoidal regression model [6] was fitted to estimate the phase difference in months (converted to days by multiplying by 30) between changes in each of the meteorological

Corresponding author and reprint requests: R. W. Thomsen, Department of Clinical Epidemiology, Aarhus University Hospital, AHSIC, Sdr. Skovvej 15, DK-9000 Aalborg, Denmark
E-mail: r.thomsen@rn.dk