Characterisation of a synergohymenotropic toxin produced by *Staphylococcus intermedius*

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Abstract Staphylococcal synergohymenotropic (SHT) toxins damage membranes of host defence cells and erythrocytes by the synergy of two secreted and non-associated proteins: class S and class F components. Whereas Panton-Valentine leucocidin (PVL), γ-hemolysin and Luk-M from *Staphylococcus aureus* are members of this toxin family, a new bi-component toxin (LukS-I + LukF-I) from *Staphylococcus intermedius*, a pathogen for small animals, was characterised and sequenced. It is encoded as a *luk-I* operon by two cotranscribed genes, like PVL. LukS-I + LukF-I shares a strong leukotoxicity of various PMNs, but only slight haemolytic properties on rabbit erythrocytes. When intradermally injected into rabbit skin, a 100 ng dose caused acute inflammatory reaction leading to tissue necrosis. The new SHT seemed to be largely distributed among various *Staphylococcus intermedius* strains.

Key words: Synergohymenotropic toxin; *Staphylococcus intermedius*; LukS-I; LukF-I; Leukotoxicity; Dermonecrosis; Virulence factor

1. Introduction

Staphylococcus intermedius (*S. intermedius*) is a coagulase-positive staphylococcus [13], whose ecology remains unclear. However, it is recognized as a veterinary pathogen which is essentially isolated from idiopathic superficial or deep recurrent skin infections of dogs and dog gingivitis [2,27]. In humans, the germ has been reported to be identified in infected varicose ulcers of elderly patients after dog bites in wounds and anterior nares [27,28]. Khambaty et al. [16] also reported an outbreak after food poisoning of butter blend. Infections and recurrent infections of dogs and food poisoning were the source of epidemiological studies [2,15,16,27].

Phenotypic and immunological detections indicated that *S. intermedius* produces coagulase, enterotoxin A and C, β- and δ-hemolysins, protein A and proteolytic enzymes in common with *Staphylococcus aureus* (*S. aureus*) [1,3]. However, no molecular characterisation of such compounds has been reported to date, except for the thermonuclease [4].

In the case of *S. aureus*, a closely related bacterium at the phenotypic level, Panton-Valentine leukocidin (PVL)-producing strains were recently reported to be strongly associated with human furuncles [8,9,19] which can lead to recurrent skin primary infections. The latter toxin (LukS-PV+LukF-PV) was recently cloned and sequenced [20]. It forms, with γ-haemolysin (HlgA, HlgB, HlgC) from *S. aureus* [7], a family of toxins acting by the synergy [12,33] of two secreted but non-associated proteins of class S (LukS-PV, HlgA, HlgC) and class F (LukF-PV, HlgB). The target cells of these toxins are host defence cells such as neutrophils, monocytes and macrophages, and also erythrocytes according to the proteins [20]. This family of toxins was termed synergohymenotropic (SHT) toxins because of the synergistical aspect of class S and class F components which may be intergangeable [20] and because they are membrane damaging toxins [6,10].

Proteins of class S are known to bind at first to the membranes of sensitive cells [6] prior to the secondary binding of class F components, which then allows the expression of biological activity. The biological activity results in the formation of pores of divalent ion size [10], thus activating neutrophils at sub-lytic levels which are responsible for neosynthesis and secretion of inflammatory mediators such as leukotrien B4, interleukine-8, and histamine [14,17,23]. Inflammatory lesions can be observed after the intradermal injection of toxins to rabbits where PVL is responsible for acute necrosis [9,32].

Since *S. intermedius* is mostly involved in skin infection of small animals, the aim of this work was to characterise such a SHT-related toxin, and to establish its main characteristics.

2. Materials and methods

2.1. Bacteria and animals

Among the 53 strains of *Staphylococcus intermedius* tested in this study, 20 were provided by Prof. R.P. Allaker (Dental School of London), 12 by Dr. Wegener (National Veterinary Laboratory, Copenhagen), 16 by N. El Solh (Institut Pasteur. Paris), and 5 by Y. Brun (Lyon). 44 of these strains were from canine origin, 8 from human origin, 1 from pigeon origin. *Escherichia coli* NM 522 [*sup*E *thi hsd*R Δ(*lac-proAB*) F′ (*proAB lacI*^{q} ZM15)] was used as the host strain for the recombinant pUC19 plasmid [34].

2.2 Protein purification

From a fresh culture of *S. intermedius* strain IBS 62 (or ATCC 51874) on sheep blood agar plate, one colony was grown for 6 h at 37°C with shaking in 3 ml of CCY-modified medium [11]. A 60 μl inoculum was then used to inoculate 20 ml of CCY-modified medium in each of the twelve 2-liter Erlenmeyer flasks containing a dialysis bag with a cut off of 6–8 kDa (Visking) filled with 120 ml of the same buffer. After overnight growth, the culture supernatant was centrifuged for 10 min at 15,000 × *g* at 4°C and then dialysed against 50 mM MES buffer, pH 6.0. Each eluted fraction from chromatographies was tested for leukotoxicity as pairs, and also alone in combination with LukS-PV and LukF-PV. The first procedure was designed for a compound which was able to lyse the human glass-adherent leukocytes when combined with LukS-PV. The dialysed solution was then chromatographed through a Mono S FPLC (Pharmacia-Uppsala, Sweden) with a linear gradient of 0 to 500 mM LiCl in MES 60 mM pH 6.0, and fractions containing proteins which were eluted between from 0 to 340 mM LiCl were collected. This protein solution was adjusted to 0.8 M $(NH_4)_2SO_4$ with 50 mM phosphate buffer pH 7.0, 3 M $(NH_4)_2SO_4$ and chromatographed through an Alkyl Superose FPLC (Pharmacia-Uppsala, Sweden) with a linear gradient from 800 mM to 0 mM $(NH_4)_2SO_4$.

Another protein component, which when combined with LukF-PV induced leukotoxicity upon the same cells, was purified by a similar

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procedure. Briefly, the culture supernatant was chromatographed through a MonoS FPLC in 50 mM Na-phosphate pH 7.0 with a linear gradient ranging from 100 mM to 600 mM NaCl. The protein fraction of interest was then adjusted to 1.5 M $(NH_4)_2SO_4$ with 50 mM phosphate buffer pH 7.0, 3 M $(NH_4)_2SO_4$ and chromatographed through an Alkyl Superose FPLC (Pharmacia-Uppsala, Sweden) in a 50 mM phosphate buffer pH 7.0, with a linear gradient of $(NH_4)_2SO_4$ ranging from 1500 mM to 900 mM.

Purified protein fractions were further analysed by SDS-PAGE [18] using the PHAST System (Pharmacia-Uppsala, Sweden). Characterisation of the purified proteins was first carried out by N-terminal sequencing (Laboratoire de Génétique Moléculaire des Eucaryotes-Strasbourg, France).

2.3. Nucleic acids methods

Total DNA from *S. intermedius* strains was prepared from a 15 ml overnight culture in 2 × TY medium as reported previously [25] for these strains except that protoplasts were obtained with a two-fold increased (20 U/ml) concentration of recombinant lysostaphin (Applied Microbiology Inc, New York, USA), and also (10 mg/ml) lysozyme (Appligene, Strasbourg-France). For the cloning of *luk-I*, partial DNA libraries were constructed by selecting and ligating *Acc*I-restricted DNA fragments ranging from 4.2 to 6.0 kb and *Dra*I-restricted DNA fragments ranging from 1.7 to 2.3 kb, into the pUC19 plasmid linearised by *Acc*I or *Sma*I, respectively. After transformation of *E. coli* NM522, recombinant clones were screened as described previously [25] using a 5'-labelled degenerate oligonucleotide probe. This 33-mer oligonucleotide (5'-GAAGAAATC/TGGAGAAGGAGCA/C/TCAAA-TC/TATC/TAAA-3') was deduced from the underlined sequence (ANTIEEIGEGAQIIK) of the N-terminal sequence of purified LukS-I, taking into account the codon usage for exoproteins from *S. aureus*.

Nucleotide sequences were determined by the dideoxy chain termination method [22] using T7 DNA polymerase [29] on double stranded DNA. The primers used initially were the universal primer and the cloning oligonucleotide and then those deduced from previous sequencing steps. Total RNA from *S. intermedius* strain ATCC 51874 was prepared as described earlier [25]. DNA–DNA [24] and RNA–DNA [30] hybridizations were performed with a 1985 bp nick-translated *Dra*I-*Dra*I DNA fragment covering both *lukS-I* and *lukF-I* in 6 × SSPE, 5 × Denhardt's solution, 0.5% × SDS at 60°C for 16 h. Filters were then washed twice in 1 × SSPE, 0.5% × SDS at 50°C for 15 min. Sequence analysis was carried out using DNAStar software (DNAStar Ltd, London, UK).

2.4. Leukocytolytic and haemolytic activity of SHT toxins

The method used for the evaluation of Luk-I and the heterologous SHT toxins combined with LukS-I or LukF-I was as described previously by Finck-Barbançon et al. [11], and was performed with human and canine glass-adherent leukocytes. However, since it has been reported [6] that LukS-PV binds with K_d = 6 nM to the membranes of target cells prior to the interaction with LukF-PV, limiting amounts of class S components (not exceeding 1 nM) were tested in the presence of an excess of class F components (50 ng or 1.55 pmol/20 μl) in 9.55‰ PBS-Dulbecco without Ca^{2+}- or Mg^{2+}-0.5% gelatin.

Haemolytic activity of the combination of LukS-I and LukF-I with other SHT components was determined as reported previously [20] on human, rabbit, and dog erythrocytes, in the presence of an excess of 125 ng of LukF-I or another class F component, per assay.

2.5. Activity of SHT toxins in a rabbit skin model

Shaved New Zealand rabbits (10 weeks old) were injected intradermally with 50 μl of apyrogenic physiological medium containing equal amounts (3, 30, 100, 300, 1000, 3000, 10000 ng) of one class S- and one class F-related component. Assays were performed in duplicate. Macroscopic observations of the lesions were recorded after 0 h, 4 h, 24 h, 48 h and lesions were biopsied under general anaesthesia induced by intramuscular injection of 250 μl of reconstituted Zoletil-100 (Reading Laboratories, Carros-France). Skin biopsies were fixed in Bouin solution, paraffin-embedded, cut in 4 mm-thick sections, then stained with hematoxylin-eosin-safran-astra blue. The tissue sections were examined in a 'blind' fashion.

3. Results

3.1. Characterisation of a new SHT toxin

A series of ten independent *S. intermedius* strains was grown overnight as described previously for the production of PVL and γ-hemolysin. The culture supernatants were then assayed for their leukotoxicity on human glass-adherent blood cells, by immunodiffusion and immunoblotting using polyclonal anti-LukS-PV or anti-LukF-PV affinity-purified antibodies. Culture supernatants appeared to be leukotoxic for human cells up to a 1/2000 fold dilution. The morphological changes of the supernatant-treated leukocytes were the same as those observed previously by phase contrast microscopy of PVL-treated cells [11,12]. Immunodiffusion and immunoblots revealed the presence of LukF-PV cross-reacting material (Fig. 1) with an apparent molecular mass of 31 kDa, but no protein was detected with LukS-PV polyclonal affinity-purified antibodies.

The attempting to purify the leukocytolytic compound produced by *S. intermedius* ATCC 51874 by the previous purification protocol for PVL and γ-hemolysin from *S. aureus* ATCC 49775 allowed the purification of a class F-related protein which was first recovered through the MonoS FPLC, and further eluted at 150 mM $(NH_4)_2SO_4$ from the Alkyl-Superose FPLC. A class S-related protein was similarly obtained which was eluted at 350 mM NaCl on MonoS FPLC and 1050 mM $(NH_4)_2SO_4$ on Alkyl Superose FPLC. The yield of 2 mg of the purified class S-related and class F-related protein by liter of crude culture supernatant may reflect the yield of production, as well as the efficiency of purification. The latter was greatly improved for the purification of the class F-related component using 50 mM MES pH 6.0 buffer and LiCl for the cation exchange chromatography. Protein fractions of interest were dialysed against 9.55‰ PBS-Dulbecco without Ca^{2+} or Mg^{2+} and stored frozen at −80°C to 1 A_{280} unit. SDS-PAGE of the purified proteins showed apparent molecular masses of 32 kDa and 35 kDa for the class S- and the class F-related protein (Fig. 1), respectively.

Table 1
Leukotoxic specific activities on human PMN cells of each possible combination of LukS-I and LukF-I with SHT components

Specific activity (U/mg)[a±b] when paired with:							
	LukF-I[b]	HlgB	LukF-PV	LukS-I	HlgA	HlgC	LukS-PV
LukS-I	9.7×10^7 ($\pm 5 \times 10^6$)	9.7×10^5 ($\pm 5 \times 10^4$)	2.9×10^5 ($\pm 10^4$)	$< 10^4$	$< 10^4$	$< 10^4$	$< 10^4$
LukF-I	$< 10^4$	$< 10^4$	$< 10^4$	9.7×10^7 ($\pm 5 \times 10^6$)	1.2×10^6 ($\pm 10^5$)	3.5×10^5 ($\pm 10^4$)	$< 10^4$

[a]Values indicate the means of quadruplicate assays (± standard deviation). One unit is defined as the limiting amount of the class S component in the presence of an excess of class F component contained in dilutions producing 100% of the morphological changes in 10^5 glass-adsorbed PMN cells.

[b]Each combination was tested in the presence of an excess of the class F component (50 ng).

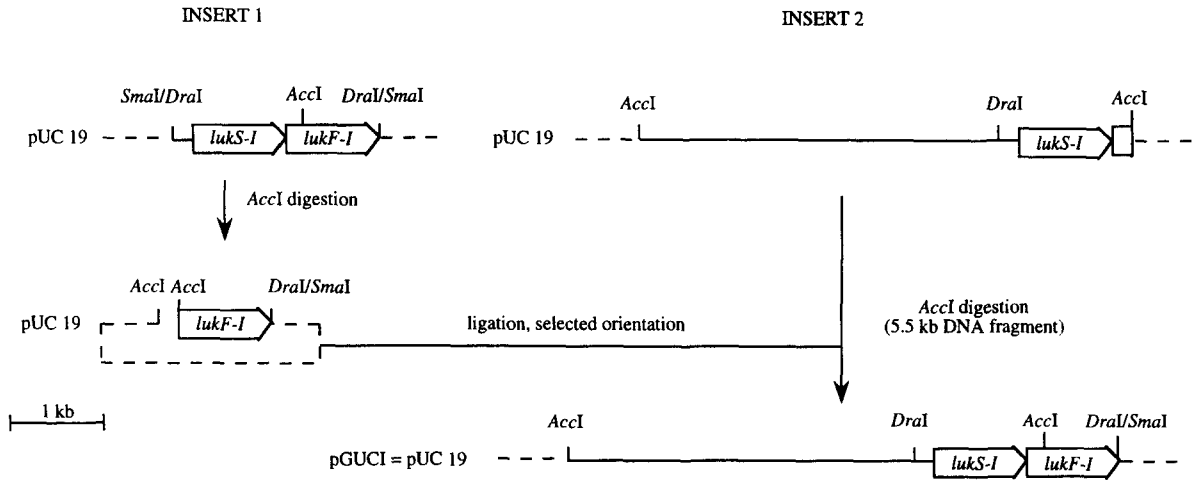

Fig. 1. (A) SDS-PAGE analysis on PHAST system of the two components of the SHT produced by *Staphylococcus intermedius*. Lane 1: 0.2 μg of LukS-I. Lane 2: 0.2 μg of LukF-I. Lane 3: 1 μg of molecular weight marker (Pharmacia, Uppsala, Sweden). (B) Immunoblot analysis of the immunoreactivity of LukS-I and LukF-I with anti-LukS-I (lanes 1 and 2) and anti-LukF-I (lanes 3 and 4) affinity-purified rabbit antibodies. Lane 1: 0.5 μg of purified LukS-I. Lane 2: culture supernatant of ATCC 51874. Lane 3: 0.5 μg of purified LukF-I. Lane 4: culture supernatant of ATCC 51874.

3.2. *Cloning and sequencing of the SHT-encoding locus from* S. intermedius

The determination of the N-terminal extremities of the class S-related protein (ANTIEEIGEGAQIIK) and the class F-related protein (ANQITPVSEKKVDDKIT) confirmed their homology with previously sequenced proteins [7,20,25], which were called LukS-I and LukF-I, respectively. A degenerate oligonucleotide probe deduced from the N-terminal sequence of the class S-related protein was first tested for hybridisation with endonuclease-restricted total DNA of *S. intermedius* ATCC 51874 (data not shown). Four (*Bam*H1, *Eco*R1, *Eco*RV, *Pvu*II) out of the nine enzymes (*Acc*I, *Cla*I, *Dra*I, *Pst*I, *Pvu*II) tested gave hybridising DNA fragments longer than 8.0 kb, whereas the detected *Dra*I-*Dra*I and *Acc*I-*Acc*I DNA fragments were relatively short (< 3.0 kb). Two partial DNA libraries were constructed in pUC19 and recombinant clones were screened using the degenerate oligonucleotide probe. From the two different selected inserts, the nucleotide sequences demonstrated that the *Dra*I-*Dra*I DNA fragment covers two open reading frames (ORFs) and that one extremity of the *Acc*I-*Acc*I DNA fragment ended within the second ORF. A long insert including the two ORFs and upstream sequences was then created (Fig. 2) in pUC19 by first linearising the plasmid obtained from the screening of the *Dra*I library, at the *Acc*I site and excluding the 1.0 kb DNA fragment. The linearised plasmid was ligated with the 5.5 kb *Acc*I-*Acc*I insert from the plasmid obtained from the screening of the *Acc*I library. After selecting the in frame orientation, the resulted plasmid (pGUCI) was used to transform *E. coli* NM522. Crude extracts of recombinant bacteria possessed a leukotoxic activity on human glass-adherent leukocytes after to be 1/500 fold diluted.

A 2662 bp long nucleotide sequence of the pGUCI insert was determined (Fig. 3). The first ORF extended from nucleotide 742 to 1674 and encoded a 310 amino acid long protein having a molecular mass of 35,056 Da. This ORF harboured 70.2%, 71%, 70.1%, 67.8% identity with other class S proteins *hlgA*, *hlgC*, *lukS-PV*, and *luk-M*, respectively. Predicted transcription signals were found 19 and 32 nucleotides upstream to the ATG initiation codon of *lukS-I* and were designated as −35 and −10 consensus sequences. A putative ribosome binding site (rbs) was also found 8 bases upstream to the ATG. This rbs sequence is often encountered for staphylococcal genes.

The second ORF was located one T downstream the stop codon of *lukS-I*, and extended from nucleotide 1676 to 2656, as was observed for the other co-transcribed genes encoding staphylococcal leukotoxins [7,20,25]. This ORF encodes a 326 amino acid-long protein which has a molecular mass of 36,600 Da. The corresponding nucleotide sequence had 76.1% and 72.3% identity with ORFs with other class F components, *hlgB* and *lukF-PV*, respectively, whereas it has only 30% identity with ORFs encoding class S-related proteins. This gene was named *lukF-I*. No ORF was observed in the 740 bp preceeding *luk*S-*I*. Therefore, this locus is organised like *luk-PV* encoding PVL, rather than *hlg* encoding γ-hemolysin and comprising three genes. No further ORF was found in the latter part of sequence. This nucleotide sequence was deposited in the EMBL/Genbank Database Library with the number X79188.

3.3. *Co-transcription of lukS-I and lukF-I*

Northern blot analysis (Fig. 4) of the hybridization of a nick-translated 1985 bp *Dra*I-*Dra*I DNA fragment covering both ORFs with total RNA from a culture (early stationary phase growth) of *S. intermedius* strain ATCC 51874 showed that *lukS-I* and *lukF-I* are co-transcribed as a single 1950 base-long mRNA. Specific mRNA corresponding to as little as 4×10^5 CFU (Fig. 4, lane 4) was detectable in this experiment.

3.4. *Peptide sequences and comparison*

From the peptide sequence deduced from *lukS-I*, the determined N-terminal peptide sequence was found 29 amino acids after the first residue. These 29 residues correspond to the signal peptide cleaved during the secretion of the mature protein and show a usual structure when compared with other staphylococcal signal peptides. Therefore, the mature protein LukS-I is 281 residues long with a molecular mass of 32,056 Da for a calculated $\varepsilon = 38{,}120 \pm 5\%$ $M^{-1} \cdot cm^{-1}$ and a calculated pI = 9.34 which were very close to those of other class S proteins. It contains 37 basic, 30 acidic, 90 hydrophobic and 85 polar residues.

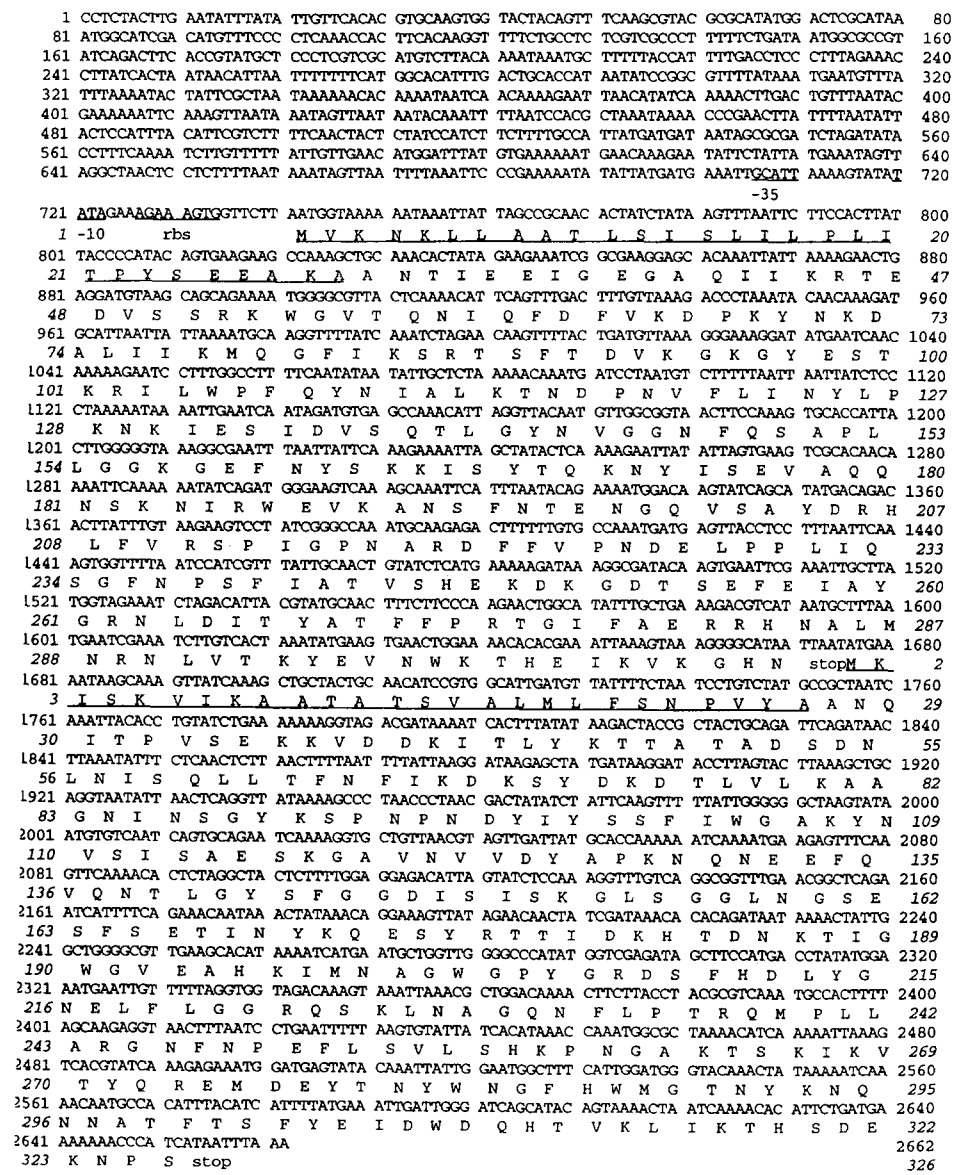

Fig. 2. Schematic reconstruction of *luk-I* from the two selected *Acc*I-*Acc*I and *Dra*I-*Dra*I inserts.

From *lukF-I*, the previous determined N-terminal peptide sequence was found 26 amino acids after the first residue. The 26 amino acids correspond to the signal peptide which contains basic residues at the N-terminal extremity, followed by hydrophobic amino acids, generally encountered in staphylococcal secreted proteins. The secreted LukF-I would be 300 amino acids long with a molecular mass of 33,862 Da for a calculated $\varepsilon = 57{,}180 \pm 5\%\ M^{-1} \cdot cm^{-1}$, and a calculated pI = 7.54 which differs from those of other class F proteins, being more alkaline. The mature LukF-I would contain 32 basic and 32 acidic, 82 hydrophobic and 109 polar amino acids.

When compared with homofunctional components of other SHT, LukS-I harboured identity in the range of 65% to 67% with LukS-PV, HlgA and HlgC, and Luk-M whereas LukF-I has 72% and 73% identity with LukF-PV and HlgB, respectively. Amino acid differences were distributed throughout the sequences.

3.5. Leukotoxic and haemolytic activities of LukS-I and LukF-I

Since each SHT component of class S and class F components exhibited synergistic activity [12,33], and since class S components bind membranes prior the interaction of the class F components [6], LukS-I and LukF-I were tested in combination (Table 1) with staphylococcal class F and class S components, respectively. The couple LukS-I + LukF-I has a very high biological activity of 9.7×10^7 U/mg on human glass-adherent blood cells which is comparable [20] with that of PVL (LukS-PV + LukF-PV). Despite being 100-fold less effective than LukS-I + LukF-I, LukS-I is still able to induce significant leukotoxicity when combined with class F components produced by *S. aureus* such as HlgB and LukF-PV. Only the combination of LukS-PV + LukF-I had no significant effect nor did components belonging to one class. However, LukF-I was able to induce leukotoxicity in combination with HlgA and HlgC, the two S-related components from γ-haemolysin. LukS-I + LukF-I

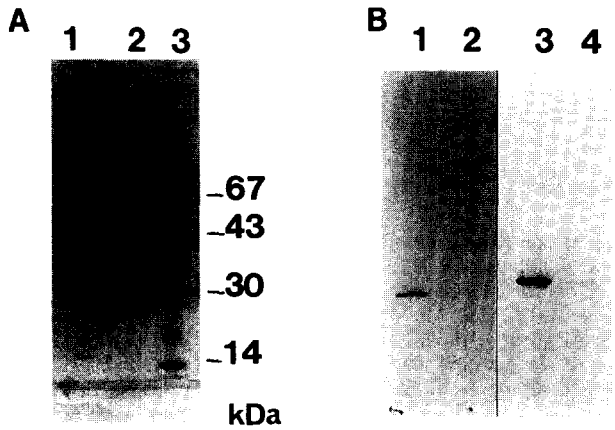

Fig. 3. Nucleotide sequence of the cotranscribed *lukS-I* and *lukF-I* constituting the *luk-I* operon. Predicted transcription consensus sequences (−35 and −10), and ribosome binding site (rbs) are underlined upstream *lukS-I* which extends from nucleotide 742 to 1674. The other ORF encoding *lukF-I* extended from position 1676 to 2656. Peptide sequences of LukS-I (310 residsues) and of LukF-I are indicated under nucleotide sequences. Probable signal peptides are also underlined.

also showed a high toxicity (3.8×10^7 U/mg of LukS-I) on canine glass-adherent blood cells, whereas other combinations appeared ineffective on these cells.

The haemolytic activity of LukS-I + LukF-I was quite low (5×10^3 U/mg) when tested on rabbit erythrocytes, if compared to the couples HlgA + HlgB and LukS-I + HlgB (5×10^7 U/mg), but the couple HlgA + LukF-I had no more activity. The other combination of SHT components with LukS-I or LukF-I did not exhibit significant haemolytic activity. The above couples also did not harbour any significant haemolytic activity ($< 5 \times 10^2$ U/mg).

3.6. Dermonecrotic activity of LukS-I and LukF-I

Intradermal injections of LukS-I + LukF-I in rabbit skin were performed as for PVL in a previous work [9]. For injections of the physiological solution or the 10,000 ng dose of proteins alone, there was a small erythema which disappeared within 48 h. At the dose of 3 ng of both components, a slight erythema was observed 24 h after injection which persisted until 48 h. At the 30 ng dose, large and infiltrated erythema was obtained in all cases at 24 h and 48 h after injection. At 100 ng doses or higher, there was eshar formation 24 h after injection with dose-dependent necrosis at 48 h. Lesions healed within 10 days except for doses of 1000 ng and greater. Histological changes were as observed after injection of PVL [9] and were chronologically: vasodilatation, diapedesis, perivascular infiltration, leukocytoclasis, vascular and tissue necrosis arround the site of injection.

3.7. Distribution of genes encoding lukS-I and lukF-I in Staphylococcus intermedius

All of the 51 total DNAs isolated from strains of *Staphylococcus intermedius* of different origin hybridised with the *Dra*I-*Dra*I *lukS-I/lukF-I* probe. As shown in Fig. 5, two different length of *Pst*I-*Pst*I DNA fragments may carry the genes encoding LukS-I and LukF-I, indicating two possible genetic arrangements in the vicinity of the corresponding ORFs. However, a majority (42 strains) of total DNAs harboured a genetic location of *luk-I* similar to that of strain ATCC 51874. No typical association was evident among the *S. intermedius* strains for either location. Culture supernatants of all strains appeared strongly leukotoxic and had biological activity even when 1/3000 fold diluted in PBS-gelatin. Immunodiffusion assays gave similar results as strain ATCC 51874.

4. Discussion

Although *S. intermedius* can be considered as a close bacteria with many phenotypes in common with *S. aureus*, it seems a more adapted pathogen to small animals. The two species may have diverged recently in the evolution and may have conserved the same process of pathogenicity against their respective hosts. Effectively, several virulence factors appeared to be encountered in the two bacteria. Here is the molecular characterisation of a leucotoxin from *S. intermedius* which is related with a recently described family of toxins produced by *S. aureus* [5,7,20,25], and different class S components may be interchangeable in order to give biological activity when combined with class F components.

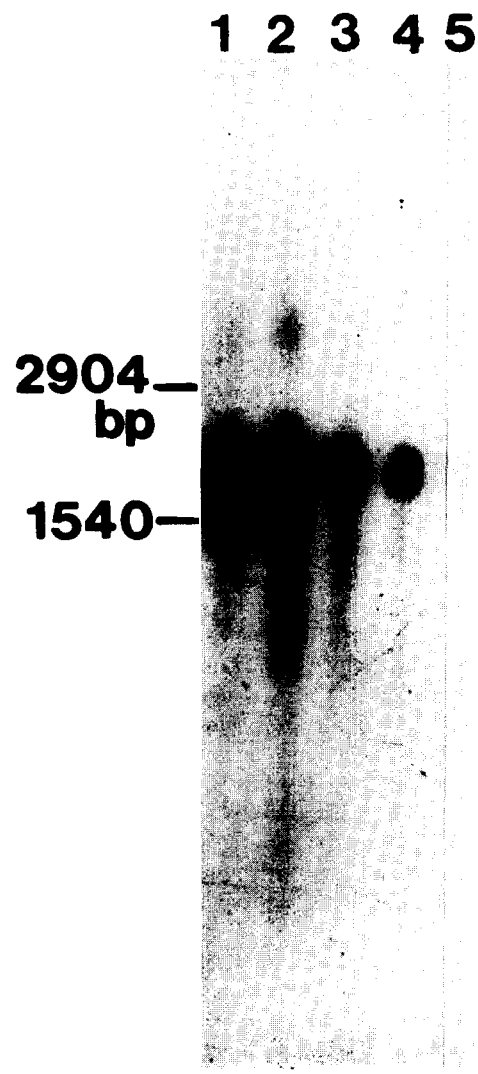

Fig. 4. Northern blot analysis evidenced the cotranscription of *lukS-I* and *lukF-I*. Lanes 1 to 5: Total RNA from 4×10^8, 4×10^7, 4×10^6, 4×10^5, 4×10^4 CFU, respectively, was hybridised to a nick-translated *Dra*I-*Dra*I DNA fragment covering both *lukS-I* and *lukF-I*.

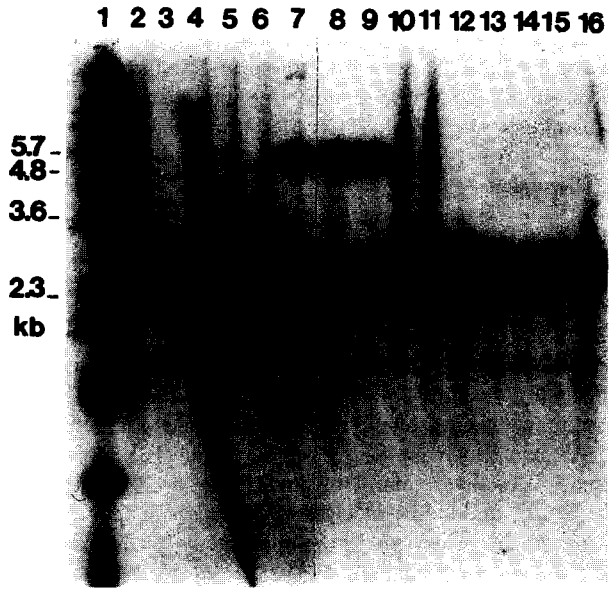

Fig. 5. Southern analysis showed two genetic arrangements for *luk-I* in the genome from *Staphylococcus intermedius*. Lane 1: 5′-labelled DNA fragment from total DNA of bacteriophage λ hydrolysed with *Bst*EII. Lanes 2 to 16: DNA–DNA hybridisation of total DNAs hydrolysed with *Pst*I from 19 of the 51 *Staphylococcus intermedius* strains with the *Dra*I-*Dra*I *luk*S-I/*luk*F-I probe.

As earlier mentioned, some consistent differences exist between Panton-Valentine leucocidin and gamma-haemolysin at the molecular level, but also concerning their genetic organisation, their gene distribution among clinical strains, their cell specificity, and their possible association to a clinical syndrome. The sequenced toxin produced by *S. intermedius* shares several aspects of SHT toxins since it is encoded by two cotranscribed genes separated by only one base. Furthermore, the gene products which are secreted separately acquired biological activity at surface of target cells only when combined each other or with heterologous components from other staphylococcal SHT toxins. The new member of the toxin family which has more or less common epitopes with other toxins, has common characters with γ-haemolysin because it is largely spread in independant strains of *S. intermedius*, and also with PVL since it is encoded by only two consecutive ORFs. LukS-I + LukF-I is highly leucotoxic on human and also on canine PMNs, but despite a slight activity on rabbit erythrocytes, it is not significantly hemolytic on cells from various mammalians. The latter properties may reflect differing specificity on target cells surface which is currently being investigated. Moreover, the *S. intermedius* leucotoxin has a sequence structure which is as divergent from γ-haemolysin as PVL, but which may have common tridimensional domains designing the functional basis of such compounds.

In conclusion, the new toxin which is the first characterised one from *S. intermedius* strengthens the notion of a toxin family produced by some of *Staphylococcus* species. Whereas one of the SHT toxins seemed to have a role in the outcoming of a clinical syndrome [8,9], the conserved properties of these toxins in several pathogenic bacteria suggest that SHT toxins are important virulence factors whose precise role remains to be determined during infections.

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