Characterization of a new cytotoxin that contributes to Staphylococcus aureus pathogenesis

Ashley L. DuMont
Department of Microbiology
New York University School of Medicine
522 First Ave, Smilow Research Bldg., Room 1006
New York, NY 10016
Phone # (212)-263-9214
Fax # (212)-263-9180
ashley.dumont@nyumc.org

Tyler K. Nygaard
Department of Veterinary Molecular Biology
Montana State University
P.O. Box 173610
Bozeman, MT 59717
Phone# (406) 994-7184
Fax # (406)-994-4303
draagynfly@hotmail.com

Robert L. Watkins
Department of Veterinary Molecular Biology
Montana State University
P.O. Box 173610
Bozeman, MT 59717
Phone# (406) 994-7184
Fax # (406)-994-4303
pv2robwatkins@hotmail.com

Amanda Smith
Department of Microbiology
New York University School of Medicine
522 First Ave, Smilow Research Bldg., Room 1006
New York, NY 10016
Phone # (212)-263-9214
Fax # (212)-263-9180
amanda.smith@nyumc.org

Lina Kozhaya
Department of Microbiology and Pathology
New York University School of Medicine
522 First Ave, Smilow Research Bldg., Room 1007
New York, NY 10016
Phone # (212)-263-9225
Fax # (212)-263-9180
lina.kozhaya@nyumc.org
Barry N. Kreiswirth
Public Health Research Institute Center
UMDNJ - New Jersey Medical School
225 Warren Street
Newark, NJ 07103
Phone# (973) 854-3240
Fax # (973)-854-3241
kreiswba@umdnj.edu

Bo Shopsin
Department of Medicine Division of Infectious Diseases
New York University School of Medicine
540 First Avenue, 2nd Floor, Lab 1, Skirball Institute
New York, NY 10016
Phone # (212)-263-9085
Fax # (212)-263-9180
bo.shopsin@med.nyu.edu

Derya Unutmaz
Department of Microbiology and Pathology
New York University School of Medicine
522 First Ave, Smilow Research Bldg., Room 1011
New York, NY 10016
Phone # (212)-263-9203
Fax # (212)-263-9180
derya.unutmaz@nyumc.org

Jovanka M. Voyich
Department of Veterinary Molecular Biology
Montana State University
P.O. Box 173610
Bozeman, MT 59717
Phone# (406) 994-7184
Fax # (406)-994-4303
jovanka@montana.edu

Victor J. Torres
Department of Microbiology
New York University School of Medicine
522 First Ave, Smilow Research Bldg., Room 1010
New York, NY 10016
Phone # (212)-263-9232
Fax # (212)-263-9180
victor.torres@nyumc.org
**Fig. S1. PCR confirmation of the deleted loci.** PCR amplification of the *lukA*, *lukE*, and *hlgC* genes from the *S. aureus* Newman wildtype (WT) strain and the isogenic mutant strains using specific primer pairs for the individual genes. *lukA* was amplified with primers VJT243 (5'-gggGAATTCTTATCCTTTTATAAGGTTTTATGTC-3') and VJT199 (5'ggggCTCGAGATGAAAAATAAAAAACGTGTTTAATAGC-3'), *lukE* was amplified with primers VJT242 (5'5'-gggGAATTCTTAAATTATGTGCCTTTTCAGTTACATTTCCCAATTTCACTCC-3') and VJT198(5'-ggggCTCGAGATGTTTAAGAAAAATGTTAGC-TGCAA-3'), and *hlgC* was amplified with VJT241 (5'-gggGAGCTCTTACATTCTGTTCACATTGATTTTAC-3') and VJT200(5'-ggggCTCGAGATGCTTTAATAAAATATTAACTACT-CAACTT-3').
Fig. S2. Exoprotein profiles of *S. aureus* strain Newman WT and isogenic mutants. Coomassie blue staining of trichloroacetic acid precipitated *S. aureus* culture supernatants from late exponential/early stationary cultures of strain Newman WT and the isogenic mutants (Δhla, Δhlg, ΔlukED, and ΔlukAB). Three individual colonies from each strain were subcultured and stained for exoprotein production.
Fig. S3. Phylogenetic tree of amino acid relatedness of LukA, LukB, and other staphylococcal leukotoxin S- and F- subunits. Amino acid sequences of the mature-secreted toxins were obtained from NCBI and the sequence alignment was performed by a Clustal W method using the MegAlign tool of DNASTAR Lasergene 8.
Fig. S4. Culture filtrate from the LukAB-negative strain remains non-cytotoxic after 24 hr. Intoxication of PMN-HL60 cells with various dilutions of culture filtrate from the WT strain Newman and the isogenic mutants for 24 hrs. Cell viability was monitored using CellTiter, where cells treated with medium were set at 100% viable. Results represent the average of triplicate samples ± S.D. * indicates statistical significance from WT, \( P < 0.05 \).
Fig. S5. Levels of LukAB are associated with the cytotoxicity of *S. aureus* clinical isolates. A. Description of clinical isolates. *spa* typing based on sequence repeats in the *spa* gene to determine the evolutionary relatedness of *S. aureus* isolates. Clonal complex (CC) was deduced from the *spa*-typing. Strains broadly grouped according to their evolutionary relatedness. BK4645 and BK4910 are colonizing isolates. B. PMN-HL60 cells were intoxicated with 20% (v/v) culture filtrate from the indicated strains and cell viability was monitored with CellTiter. Results represent the average of triplicate samples ± S.D. LukB levels in culture filtrates of the indicated strains was determined by immunoblotting with an anti-LukB sera. References: (1) Roberts *et al.*, 1998 J Infect Dis 178: 164-171. (2) Shopsin *et al.*, under review. (3) Shopsin *et al.*, 2003 J Clin Microbiol 41: 456-459.