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Clones and Drones: Do Variants of Panton-Valentine Leukocidin Extend the Reach of Community-Associated Methicillin-Resistant *Staphylococcus aureus*?

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(See the article by O'Hara et al., on pages 187–94.)

The most important toxic fraction in severe human lesions is the leucocidal . . . the hemolysin and necrotoxin vary closely together with no relation to the leucocidin.

—Panton and Valentine, 1932 [1]

In 1932, Philip N. Panton and his colleagues, as they studied bacterial toxins at the London Hospital, could not have anticipated the scientific revolution coming in microbial genomics. It is ironic, then, that some of the genes encoding those toxins Panton and colleagues discovered well before the midpoint in the 20th century would now be contributing to our knowledge of how bacterial pathogens arise and evolve [2]. Even then, the work of these toxin hunters was specific enough to suggest that some of the toxins, in light of their cellular targets, were separate entities. Their term, "staphylococcal toxin," included the various toxigenic effects produced by staphylococcal "constituents" that were either hemolytic (hemolysins), necrotic when injected subcutaneously (necrotoxins), destructive for phagocytes when staphylococcal suspensions were diluted 1:16 (leukocidins), or fatal for rabbits after intravenous injection (lethal toxin) [1].

In the early 1930s, there were no therapeutic antimicrobials and no penicillin resistance in staphylococci, let alone the resistance to semisynthetic penicillins introduced some 30 years later [3]. For that reason, in the early part of the last century, the ultimate intent of staphylococcal research was to develop specific antisera against suspected toxins in an attempt to counter the ineffective therapy of the vaccines of the era, which were composed primarily of bacterial debris [4]. It is likely that many of the early isolates of *Staphylococcus aureus* contained the genes (*lukS-PV* and *lukF-PV*) that encode the most important leukocidin, appropriately named "Panton-Valentine leukocidin" (PVL) [5]. In fact, there were reports of antitoxins being used efficaciously to treat staphylococcal infections [4], but those studies did not continue. Monoclonal antibodies to some of the MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), on the other hand, have shown new promise for immune-based therapy [6]. Conversely, the exact role played by leukocidins such as PVL in the pathogenesis of staphylococcal infection remains controversial [7]. PVL-containing *S. aureus* continues to cause severe necrotizing community-acquired pneumonia [8], and PVL has been shown to be sufficient to cause necrotizing pneumonia when administered to mice [9].

In 2006, an extraordinary finding was reported concerning the spread in the community of a predominant strain of methicillin-resistant *S. aureus* (MRSA) termed "USA300," which displayed an easily identifiable pattern when analyzed by pulsed-field gel electrophoresis (PFGE) and elaborated PVL [10]. The "USA" designations originally published in 2003 derived from the most common PFGE patterns of US isolates of MRSA [11]. USA300 isolates arose primarily from outbreaks in correctional institutions, among athletic teams, and in nurseries [12]. Oxacillin (methicillin) resistance had historically been linked to multiple resistance determinants housed within a large, complex mobile genetic element termed "staphylococcal chromosome cassette" (SCC*mec*) [13]. In contrast, community-associated MRSA (CA-MRSA) isolates such as USA 300 usually contained a smaller, truncated SCC*mec* [14]. Community strains containing SCC*mec*

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type IV spread rapidly; by August 2004, among emergency departments in 11 US cities, 78% of isolates from 320 patients were MRSA, and 98% of these were USA300 [10]. Moreover, 98% of USA300 contained the SCC*mec* type IV and the *lukSF-PV* genes encoding the 2-component PVL, whereas genes for common staphylococcal enterotoxins and toxic shock syndrome toxin–1 were seldom present. Of MSSA isolates in the emergency-department study, 31% were also USA300 pulsotype, and 42% of these contained *lukSF-PV*. Subsequently, among patients admitted to a large public hospital in Los Angeles, both MRSA and MSSA USA300 PVL-positive strains were noted to be prominent, but there were no reliable risk factors that distinguished patients infected with CA-MRSA from those infected with CA-MSSA [15]. In Chicago from 2003 to 2005, CA-MRSA increased from 24 to 164 cases/100,000 persons, but CA-MSSA also continued to increase [16].

As this community pandemic continued, there was inevitable spread to hospitalized patients. At the Houston Veterans Affairs Medical Center during 2003 and 2004, 60% of MRSA nosocomial isolates were USA300 PVL positive [17]. In this study, typical hospital strains were unlikely to be PVL positive, as was shown subsequently to be true of specific nosocomial strains as well—namely, USA200 [18]. While USA300 continued to be prominent in the United States, it also sprung into European collections of MRSA, emerging in Denmark on several occasions between 2000 and 2005 [19]. Thus, there was something special about USA300, particularly with reference to its inclusion of *lukSF-PV* and SCC*mec* type IV and its spread and severity of disease [20].

In this issue of the *Journal,* workers from GlaxoSmithKline (GSK) extend our knowledge of PVL, its evolution, and its structure [2]. They have exploited the collection of *S. aureus* isolates from 3 GSK global clinical trials involving the use of a new topical antimicrobial, retapamulin. Most of the isolates ($n = 117$) were MSSA, reflecting different *S. aureus* population distributions in different countries. Using polymerase chain reaction, the investigators amplified the full sequence of *lukSF-PV* from 174 of 177 isolates from the combined studies. From genetic data for *lukSF-PV* alleles and from multilocus sequence typing, they constructed a statistical parsimony network. Because of some striking implications from the sequence analysis, they proceeded next to construct a model for the PVL octamer, particularly as it relates to the structure of an α-hemolysin pore former and its projected interaction with the cell membrane.

Here is what the study found. The *lukSF-PV* nucleotide sequence is highly preserved, but there were 12 sites that varied. Only 1 of those sites resulted in an amino acid change and was common. The amino acid change from histidine (H variants) to arginine (R variants) in almost all USA300 is found in a region of the LukS-PV protein. On the basis of modeling of the protein and its interaction with LukF-PV and similar proteins, this change is predicted to play a role in altering the function of the toxin, such as toxin complex diversity, number, stability, and rate of pore formation. The hypothesis that this mutation actually plays a role in the efficiency of the toxin in causing severe disease remains to be proved.

The 2 major PVL variants in the GSK clinical trials collection—namely, the H variants and the R variants—were distributed according to geography. The R variant was predominantly found in the United States, including in the USA300 clone and in the original CA-MRSA strain implicated in lethal childhood infections, USA400 (MW2) [21]. In contrast, the H variants were predominantly found in *mecA*-negative MSSA strains from other countries in Europe and South Africa. Currently, severe MSSA infections associated with PVL toxin are reported infrequently. Thus, could the apparent enhanced virulence of USA300 and USA400 result from the acquisition of a novel PVL or acquisition of a particular SCC*mec*? To understand this hypothesis better, it is important to know how *S. aureus* evolves and what might be driving this evolution.

All *S. aureus* strains have conserved components in their genome, but the population structure consists of distinct lineages (clonal complexes), and each lineage is remarkably different, especially in its surface structures that are predicted to bind to host tissues and play roles in colonization and infection [22]. In addition, a range of virulence and toxin genes move into and out of each lineage at high frequency on mobile genetic elements [23]. Mobile genetic elements include the SCC*mec* type IV element carrying methicillin resistance and the family of bacteriophages encoding LukSF-PV [24]. Evolution of *S. aureus* will occur when a mutation or acquired gene or element gives the bacterium an advantage in a specific environment. The normal habitat of *S. aureus* is the human nose and mucous membranes, and typical challenges might be exposure to antibody, innate antibacterial defenses, antibiotics, or antiseptics, such as those found in cleaning and hygiene products.

MRSA strains that are PVL positive are increasingly reported in Europe, Asia, and Australasia, and they cause severe infections [25]. Unfortunately, these isolates were not tested in the study by O'Hara et al. [2], but a rapid communication recently suggested that at least some of the PVL genes in these strains were of the H type [26]. This observation argues against the hypothesis that PVL variation to the R type contributes to the evolution of strains that cause enhanced disease. We should be careful, however, about how we interpret these data, because other factors besides staphylococcal toxins, such as innate immune modulators, may be located on bacteriophages [27].

Infections and outbreaks of disease in different countries can be difficult to compare because of variations in human populations, nasal colonization rates, antibiotic use, health care systems, and diagnostic criteria. Such variables affect this study, because the bacteria in different countries vary substantially. For example, USA300 belongs to the *S. aureus* clonal

complex CC8, and USA400 belongs to CC1. In Europe, Asia, and Australia the dominant PVL-positive MRSA belong to CC30, CC59, and CC80. Because each lineage is unique, any of these variables could play a role in disease, possibly in combination with PVL, affecting clinical outcome. Furthermore, the *lukSF-PV* toxin genes can move into and out of strains and may evolve independently of their host bacterium. Indeed, it could be that restriction barriers controlling bacteriophage spread have controlled the distribution of the *lukSF-PV* gene variants [28]. In addition, other resistance and virulence genes, including those toxins originally reported by Panton and colleagues, are also encoded on mobile genetic elements that move into and out of strains [23].

The interesting question remains why clones such as USA300 have evolved and spread so quickly, causing intense, widespread disease, especially in healthy young persons in the community. It is currently difficult to tell how USA300 emerged, and the current work suggesting entry of *lukSF-PV* into specific lineages is probably one small snapshot of the process. CC8 has become a common worldwide clone of nosocomial MRSA, MSSA, and CA-MSSA. A strain of USA300 has been sequenced, as have 2 UK CC8 strains from before 1960 [29]. There are many differences between them but few clues as to which differences are important. Evidence that any one of these traits is key to turning USA300 into a pathogen is currently not available. So, although variation in toxins such as PVL may be important, the answers to where USA300 has arisen and what makes it successful are still not clear. We cannot rule out the increasing use of antibacterials or antiseptics in the home or other lifestyle factors that may control *S. aureus* populations but have perhaps been overcome by USA300. Alternatively, younger populations may be naive to toxins (such as PVL) and fail to mount an appropriate immune response during carriage or infection.

Panton qualified for his MD degree in 1903 and worked as a clinical pathologist until he retired in 1946 [30]. He had a large private practice in pathology, and in 1914 he wrote the first edition of *Clinical Pathology*. He worked to promote many medical fields, particularly transfusion, but published only a handful of articles on bacterial toxins. Panton's partner in bacterial toxin work, F. C. O. Valentine, continued to publish articles on bacterial diseases after the landmark toxin work. As for Panton himself, his love was translational science, so he pushed hospitals to develop more effective hospital laboratories. He parlayed these skills to prepare England for the laboratory responses needed in World War II. In 1946, he was recognized with knighthood for his work in the Ministry of Health and for his war services. He died on 27 December 1950, as Sir Philip Noel Panton.

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