For CA-MRSA, how much PVL is too much?

Panton–Valentine leukocidin (PVL) is a cytolitic toxin, and it is encoded on a Staphylococcus aureus bacteriophage integrated into the bacterial genome. PVL may be the key toxin responsible for enhanced virulence of community-associated meticillin-resistant S. aureus (CA-MRSA), but this is controversial. In many bacteria, expression of toxin genes encoded on such phage is induced under stress, including antibiotics. In this issue, Wirtz et al. (2009) prove that phage induction increases expression of luk-PV mRNA dramatically. But surprisingly, they show that the clone responsible for most CA-MRSA infections in the USA has defective phage and fails to overexpress the toxin.

S. aureus is a commensal of humans, and an extremely common cause of infections ranging from mild to fatal. β-Lactam antibiotics, particularly the meticillin-related class, are widely used to prevent and treat infections, but resistance is now very common, especially in hospitals. CA-MRSA have evolved independently of typical hospital MRSA. The dominant CA-MRSA from different regions belong to genetically unrelated lineages and cause unique clinical problems. In the USA, CA-MRSA belong to lineages CC8 (called USA300, which is the dominant clone) and CC1 (USA400) and are responsible for widespread severe skin and soft tissue infection (SSSI) in healthy people, particularly those in close contact with others such as schoolchildren, athletes, prisoners, military personnel and men who have sex with men. Infections can be severe enough to require hospitalization and may cause death. CA-MRSA are spreading quickly, resulting in severe pressure on accident and emergency departments, and USA300 is becoming a major cause of hospital-acquired infection (Seybold et al., 2006). Furthermore, meticillin-sensitive S. aureus that are genetically similar to USA300 and PVL positive have also caused a dramatic increase in SSSI in the last few years (Orscheln et al., 2009). In Europe, CA-MRSA are rarer, but when they are present the dominant clone is ST80. Rare haemolytic pneumonia in children with a high mortality rate is associated with these strains. In Asia, SSSI are increasing due to CA-MRSA CC59, while in the South Pacific, SSSI caused by CA-MRSA CC30 have been prevalent for more than a decade (Tristan et al., 2007).

What makes CA-MRSA able to infect the skin of healthy hosts more successfully than ‘typical’ S. aureus? A likely candidate is the PVL toxin. luk-PV genes are present in approximately 2% of S. aureus, but in virtually 100% of CA-MRSA of all types. This epidemiological association makes the PV toxin an excellent diagnostic marker for CA-MRSA. It is a known virulence factor in haemolytic pneumonia. However, in mouse models of infection, there are conflicting data about whether PVL+ S. aureus are more virulent than PVL- strains (Voyich et al., 2006; Labandeira-Rey et al., 2007). PVL may target human neutrophils better than mouse neutrophils (Hongo et al., 2009), but at present comprehensive studies with a human SSSI model are not available. If proven to be important, PVL could be the best target for immunotherapy.

Induction of S. aureus bacteriophage can be caused by β-lactams, ciprofloxacin and trimethoprim antibiotics, as well as UV light and DNA-damaging agents, via the SOS system (Sumby & Waldor, 2003; Goerke et al., 2006a). Prophage is excised and replicated to high copy number, allowing a dramatic increase in the expression of genes encoded on the phage genome. Wirtz et al. (2009) found that induction with mitomycin increased luk-PV mRNA by 10-fold or more. Similar phage are widespread in S. aureus, as are the related S. aureus pathogenicity islands (SaPI), and phage and SaPI elements encode dozens of known S. aureus toxins which can all potentially be induced by this mechanism (Lindsay & Holden, 2006). However, the importance of this induction in disease has not been fully explored.

While Wirtz and colleagues show that many CA-MRSA clones induce luk-PV expression under stress, the most successful clone does not. The bacteriophage in USA300 is defective and cannot be induced to excise and replicate. This raises interesting questions. Is PVL necessary for spread and infection, but produced in sufficient quantities without induction? Is PVL not necessary for USA300 spread or infection? Is regulation at the post-transcriptional level more important?

A defective phage is unable to cause lysis of the parent S. aureus, which may be an advantage to the bacterium. However, Wirtz and colleagues also show that USA300 carries another prophage that can cause lysis, suggesting this advantage is not exploited. A defective phage is effectively stable in the genome, unlike many S. aureus prophage (Moore & Lindsay, 2001; Goerke et al., 2006b), and this may partly explain why luk-PV is so prevalent in the USA300 clone. But it doesn’t explain why...
fully mobilizable luk-PV phage are so prevalent in other CA-MRSA.

Overall, this study shows that USA300 fails to overexpress luk-PV under stress, suggesting phage induction is not an important regulatory pathway for toxin expression in SSSI infection. Once again, the role of this toxin in disease remains elusive.

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Shielding, a new pathogen defence mechanism against PMNs

In an article published in this issue, Alhede et al. (2009) demonstrate that the opportunistic pathogen Pseudomonas aeruginosa can react to the presence of PMNs by producing abundant quantities of rhamnose-containing glycolipid biosurfactants, the rhamnolipids (Maier & Soberon-Chavez, 2000). PMNs are phagocytic cells and are important players in the innate immune response since they produce a range of antimicrobial molecules able to kill pathogens. Rhamnolipids are known to cause fast lysis of amoebae such as Dictyostelium discoideum (Cosson et al., 2002); they have previously been shown by the same research group to cause lysis of several cellular components of the human immune system, e.g. monocyte-derived macrophages and PMNs (Jensen et al., 2007; Van Gennip et al., 2009). In P. aeruginosa, rhamnolipid synthesis is under control of the quorum-sensing (QS) system, particularly via the RhlR-RhlC C4-homoserine lactone synthase/LuxR regulator system (Williams & Camara, 2009). The control of rhamnolipid production by QS is necessary to avoid constant exposure of eukaryotic cells, including the components of the innate immune system, to these surfactants, which could lead to the triggering of a strong inflammatory response. To demonstrate the importance of rhamnolipids as a shield against PMNs, the authors tested the capacity of an rhlA mutant (unable to produce these biosurfactants) to survive in the lungs of infected mice and found a drastic reduction of colony-forming units compared to the wild-type.

Like other pathogens, P. aeruginosa is capable of forming biofilms (Parsek & Tolker-Nielsen, 2008). This is particularly the case in the lungs of cystic fibrosis patients, where P. aeruginosa forms biofilms in the thick mucus layer (Winstanley & Fothergill, 2009). In this study the authors measured the release of lactate dehydrogenase by PMNs in contact with in vitro P. aeruginosa biofilms, as an indicator of cell lysis. They demonstrated that a large fraction of the PMNs exposed to the wild-type biofilm rapidly lysed, while no PMNs lysed after exposure to the rhlA mutant, demonstrating the importance of rhamnolipid production as a strategy to aggressively respond to PMNs. However, in agreement with another study (Morici et al., 2007) Alhede et al. (2009) found that in vitro biofilms produce very little rhamnolipid, which at first seems to contradict the rapid lysis observed by the authors. In investigating this apparent anomaly, Alhede and colleagues demonstrated that exposure to PMNs acts as a signal triggering the fast production of rhamnolipids, which remain associated with the biofilm rather than being released in the surrounding fluid. This suggests that PMNs become eliminated by contact with the biofilm. This is in agreement with microscopic investigations of P. aeruginosa-infected tissue samples from the lungs of cystic fibrosis patients and chronic wounds, which suggest that PMNs stay in the periphery but do not reach inside the biofilm cell mass. Furthermore, lysis of the PMNs causes release of DNA, which is