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Panton–Valentine leukocidin antibodies for the treatment of MRSA skin infections?

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has recently caused epidemic outbreaks of community-associated (CA) skin infections. The infecting strains frequently contain the genes encoding the staphylococcal toxin, Panton–Valentine leukocidin (PVL). On that ground, the use of PVL-based vaccines has been proposed for the treatment of CA-MRSA infections, despite experimental and epidemiological evidence that does not support a major role of the PVL toxin in CA-MRSA skin disease. Hermos *et al.* show that antibodies to PVL do not protect from CA-MRSA skin infections in children, strongly suggesting that PVL-based immunization is of little benefit for this most frequent disease caused by CA-MRSA.

Keywords

drug development; MRSA; Panton–Valentine leukocidin; skin infections; *Staphylococcus aureus*

This article reviews the study by Hermos *et al.*, summarizes the key findings and discusses those findings regarding [1]:

- The proposed use of Panton–Valentine leukocidin (PVL) vaccines to treat infections by methicillin-resistant *Staphylococcus aureus* (MRSA);
- The contribution of PVL to virulence of MRSA strains.

Methicillin-resistant *Staphylococcus aureus* causes an estimated 95,000 invasive infections per year in the USA alone, of which approximately 19,000 are fatal [2]. Most of these infections occur in the hospital setting, such as in patients who are immunocompromised, undergoing surgery or have other predisposing conditions [3]. In addition, MRSA infections now also occur in healthy individuals without prior contact to hospitals. These community-associated (CA)-MRSA infections, which mostly present as a moderate-to-severe skin and soft-tissue infection (SSTI), were first observed in the late 1990s and since then have been reported from a variety of locations around the globe [4]. In the USA, the CA-MRSA epidemic is much more pronounced than in other countries owing to a specific CA-MRSA clone called USA300 [5,6]. CA-MRSA infections represent the cause of approximately 13.7% of invasive MRSA infections in the USA [2].

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Treatment of MRSA infections is difficult owing to the limited repertoire of antibiotics that can be used with efficiency. Furthermore, there are no effective vaccines for *S. aureus* infections [7]. Based on the association of PVL with CA-MRSA [8,9], it has been frequently suggested to use the PVL toxin as a vaccine or vaccine component for the prophylaxis or treatment of disease caused by CA-MRSA. There is currently an ongoing Phase I clinical trial injecting humans with a recombinant form of the LukS component of PVL, either alone or in combination with recombinant α -toxoid [101].

Summary of methods & results

Crucial information as to whether vaccines may work to prevent or treat human infection is achieved by analyzing whether circulating antibodies exist in humans and, most importantly, whether these protect from recurring infections. In their study, Hermos *et al.* analyzed antibody levels to PVL in serum samples from children (0–18 years of age) presenting with PVL-positive MRSA SSTI and compared them with children with PVL-positive invasive (non-SSTI) and PVL-negative MRSA infection, and to uninfected control subjects [1]. They found that children with primary PVL-positive MRSA infection mounted a robust anti-body response to PVL, in contrast to children with PVL-negative MRSA infections. Of note, they verified PVL toxin-neutralizing functionality of PVL antibodies and used rigid controls to correct for a series of parameters such as day of illness, age, history of prior infection and PVL status of the infecting MRSA isolate. When correcting for those factors, only a history of prior infection (MRSA or SSTI), but not the PVL status of the infecting isolate, was correlated with PVL antibody levels, which contrasts with results from previous, more limited studies [10–12]. As the most important finding of the study, neutralizing (active) PVL antibodies were elevated in children with MRSA-associated and recurrent SSTI. Therefore, Hermos *et al.* concluded that PVL antibodies do not protect from recurrent PVL-positive MRSA-associated SSTI.

Expert commentary

Even before the study by Hermos *et al.*, the use of a hypothetical PVL vaccine has been frequently criticized for two major reasons: first, PVL is only associated with CA-MRSA infections and is typically absent from hospital-associated (HA)-MRSA strains, which cause the majority of MRSA infections [2]. Thus, a vaccine based on PVL would be limited to the treatment of a minority of MRSA infections, namely those that are caused by a PVL-positive MRSA strain and require intervention with drugs (such as severe cases of CA-MRSA pneumonia). Second, several experimental studies indicated that the contribution of PVL to virulence, in particular in SSTI, is minor. PVL thus lacks what is considered by many experts to represent a key prerequisite for a vaccine target. Experimental studies on PVL's role in CA-MRSA pathogenesis are briefly reviewed in the following.

In CA-MRSA strains, resistance to methicillin-type antibiotics is combined with a degree of virulence that significantly exceeds that of traditional HA-MRSA strains [13–14]. Indeed, CA-MRSA strains are known to cause very severe and fatal infections, such as necrotizing pneumonia and fasciitis, which are not commonly seen with HA-MRSA strains [15–17]. It is believed that this increased virulence potential makes CA-MRSA strains fit to attack otherwise healthy people, while HA-MRSA lack such potential [14].

Analysis of the basis of CA-MRSA virulence has been focused on USA300 as the strain that is associated with the most severe epidemic [6] and on PVL, owing to the initial observation that the genes for PVL appeared to be associated with CA-MRSA strains [8,9]. We now know that this association is not absolute, as PVL-negative CA-MRSA strains have emerged [14,18]. Furthermore, while results from some animal infection studies reported that PVL

contributes to experimental skin or lung infection [19-21], many other studies failed to detect a role of PVL in CA-MRSA disease when investigating isogenic deletion mutants of the PVL-encoding genes [22-27] or the effect of PVL-neutralizing antibodies [28]. Results of animal studies evaluating the use of PVL for immunization were equally mixed [20,29].

The degree to which PVL impacts the severity of experimental CA-MRSA disease is still being heavily debated. Of note, limitations in study design in earlier studies have now been overcome, in particular regarding the choice of test species [29] and the use of appropriate genetics to substantiate alleged effects of PVL [27]. Recent infection studies in the rabbit as a species with high sensitivity to PVL indicate that PVL does not contribute significantly to CA-MRSA skin infections [30] [Kobayashi S, Pers. Comm.], but does appear to have an effect at high inocula in experimental osteomyelitis and severe pneumonia [31,32].

While results from animal infection models thus strongly suggest that PVL cannot be primarily made responsible for the enhanced virulence of CA-MRSA strains in SSTI, there are always limitations of using animal models to mimic human infection. It is therefore of utmost importance to confirm indications from animal experiments by well-controlled clinical and epidemiological studies. In keeping with the results of many animal studies and those of Hermos *et al.*, occurrence of PVL-encoding genes was not associated with a worse clinical outcome in complicated SSTI in a large international epidemiological study [33]. Thus, recent experimental, clinical and epidemiological findings consistently indicate that PVL does not enhance the severity of CA-MRSA skin infections. Consequently, including PVL in a vaccine for MRSA SSTI would be of little benefit.

Five-year view

Future research will need to evaluate whether PVL vaccines may be used to treat manifestations of MRSA disease, such as severe pneumonia and osteomyelitis, in which, in contrast to SSTI, PVL has shown a significant contribution to disease progression in animal infection models [31,32]. Of note, the fact that this contribution could only be demonstrated at high inocula indicates that PVL contributes to the exacerbation rather than to the establishment of those diseases. In that regard, the same researchers of the Hermos *et al.* study previously found that PVL antibodies may even increase the risk of establishing an MRSA SSTI [34]. This is most likely due to the activation of human host defenses by sublytic PVL concentrations – an effect that would protect from infection and be diminished by administering therapeutic PVL antibodies. When assessing the efficacy of PVL vaccines or antibodies, effects at low bacterial inocula should therefore always be investigated to rule out that antibodies to PVL may contribute to host susceptibility to infection. However, it appears more valuable for the development of efficient *S. aureus* vaccines to focus on toxins that are not only present in a majority of *S. aureus* strains, but also have a clearly demonstrated major impact on CA-MRSA pathogenesis, such as the phenol-soluble modulins- α peptides and α -toxin [26,30,35]. While targeting phenol-soluble modulins- α peptides may be difficult, as they are a family of small peptides with divergent structure and likely low immunogenicity, active or passive immunization with α -toxin was shown to successfully reduce CA-MRSA skin and lung infection [28,36].

Key issues

- Antibodies to Panton–Valentine leukocidin (PVL) do not protect from skin infections by community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA).
- Experimental, clinical and epidemiological evidence indicates that PVL has no major role in community-acquired MRSA skin infections.

- Application of a hypothetical PVL vaccine would be limited to rare cases of MRSA infection.
- Evaluation of vaccine formulas based on PVL should include an assessment of the effect of neutralizing PVL antibodies on host susceptibility to infection.

References

Papers of special note have been highlighted as: • of interest

1. Hermos CR, Yoong P, Pier GB. High levels of antibody to Pantón–Valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clin. Infect. Dis.* 2010; 51(10):1138–1146. [PubMed: 20946065]
2. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA.* 2007; 298(15):1763–1771. [PubMed: 17940231]
3. DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J. Clin. Invest.* 2009; 119(9):2464–2474. [PubMed: 19729844]
4. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet.* 2010; 375(9725):1557–1568. [PubMed: 20206987]
5. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet.* 2006; 367(9512):731–739. [PubMed: 16517273]
6. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N. Engl. J. Med.* 2006; 355(7):666–674. [PubMed: 16914702]
7. Otto M. Looking toward basic science for potential drug discovery targets against community-associated MRSA. *Med. Res. Rev.* 2010; 30(1):1–22. [PubMed: 19399829]
8. Gillet Y, Issartel B, Vanhems P, et al. Association between *Staphylococcus aureus* strains carrying gene for Pantón–Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet.* 2002; 359(9308):753–759. [PubMed: 11888586]
9. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Pantón–Valentine leukocidin genes: worldwide emergence. *Emerg. Infect. Dis.* 2003; 9(8):978–984. [PubMed: 12967497]
10. Brown EL, Bowden MG, Bryson RS, et al. Pediatric antibody response to community-acquired *Staphylococcus aureus* infection is directed to Pantón–Valentine leukocidin. *Clin. Vaccine Immunol.* 2009; 16(1):139–141. [PubMed: 19005019]
11. Verkaik NJ, de Vogel CP, Boelens HA, et al. Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of *Staphylococcus aureus*. *J. Infect. Dis.* 2009; 199(5): 625–632. [PubMed: 19199541]
12. Croze M, Dauwalder O, Dumitrescu O, et al. Serum antibodies against Pantón–Valentine leukocidin in a normal population and during *Staphylococcus aureus* infection. *Clin. Microbiol. Infect.* 2009; 15(2):144–148. [PubMed: 19154491]
13. Voyich JM, Braughton KR, Sturdevant DE, et al. Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. *J. Immunol.* 2005; 175(6): 3907–3919. [PubMed: 16148137]
14. Otto M. Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. *Annu. Rev. Microbiol.* 2010; 64:143–162. [PubMed: 20825344]
15. Adem PV, Montgomery CP, Husain AN, et al. *Staphylococcus aureus* sepsis and the Waterhouse-Friderichsen syndrome in children. *N. Engl. J. Med.* 2005; 353(12):1245–1251. [PubMed: 16177250]
16. Miller LG, Perdreau-Remington F, Rieg G, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *N. Engl. J. Med.* 2005; 352(14):1445–1453. [PubMed: 15814880]

17. Francis JS, Doherty MC, Lopatin U, et al. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton–Valentine leukocidin genes. Clin. Infect. Dis. 2005; 40(1):100–107. [PubMed: 15614698]
18. Diep BA, Otto M. The role of virulence determinants in community-associated MRSA pathogenesis. Trends Microbiol. 2008; 16(8):361–369. [PubMed: 18585915]
19. Labandeira-Rey M, Couzon F, Boisset S, et al. *Staphylococcus aureus* Panton–Valentine leukocidin causes necrotizing pneumonia. Science. 2007; 315(5815):1130–1133. [PubMed: 17234914]
20. Brown EL, Dumitrescu O, Thomas D, et al. The Panton–Valentine leukocidin vaccine protects mice against lung and skin infections caused by *Staphylococcus aureus* USA300. Clin. Microbiol. Infect. 2008; 15(2):156–164. [PubMed: 19154186]
21. Tseng CW, Kyme P, Low J, et al. *Staphylococcus aureus* Panton–Valentine leukocidin contributes to inflammation and muscle tissue injury. PLoS ONE. 2009; 4(7):e6387. [PubMed: 19633710]
22. Bubeck Wardenburg J, Palazzolo-Ballance AM, Otto M, Schneewind O, DeLeo FR. Panton–Valentine leukocidin is not a virulence determinant in murine models of community-associated methicillin-resistant *Staphylococcus aureus* disease. J. Infect. Dis. 2008; 198(8):1166–1170. [PubMed: 18729780]
23. Voyich JM, Otto M, Mathema B, et al. Is Panton–Valentine Leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? J. Infect. Dis. 2006; 194(12):1761–1770. [PubMed: 17109350]
24. Montgomery CP, Daum RS. Transcription of inflammatory genes in the lung after infection with community-associated methicillin-resistant *Staphylococcus aureus*: a role for Panton–Valentine leukocidin? Infect. Immun. 2009; 77(5):2159–2167. [PubMed: 19237525]
25. Olsen RJ, Kobayashi SD, Ayeras AA, et al. Lack of a major role of *Staphylococcus aureus* Panton–Valentine leukocidin in lower respiratory tract infection in nonhuman primates. Am. J. Pathol. 2010; 176(3):1346–1354. [PubMed: 20093487]
26. Bubeck Wardenburg J, Bae T, Otto M, DeLeo FR, Schneewind O. Poring over pores: α -hemolysin and Panton–Valentine leukocidin in *Staphylococcus aureus* pneumonia. Nat. Med. 2007; 13(12):1405–1406. [PubMed: 18064027]
27. Villaruz AE, Wardenburg JB, Khan BA, et al. A point mutation in the *agr* locus rather than expression of the Panton–Valentine leukocidin caused previously reported phenotypes in *Staphylococcus aureus* pneumonia and gene regulation. J. Infect. Dis. 2009; 200(5):724–734. [PubMed: 19604047]
28. Loffler B, Hussain M, Grundmeier M, et al. *Staphylococcus aureus* Panton–Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog. 2010; 6(1):e1000715. [PubMed: 20072612]
29. Bubeck Wardenburg J, Schneewind O. Vaccine protection against *Staphylococcus aureus* pneumonia. J. Exp. Med. 2008; 205(2):287–294. [PubMed: 18268041] • While focused primarily on α -toxin as a vaccine target, this study includes a direct comparison of the efficacies of anti-Panton–Valentine leukocidin (PVL) and anti- α -toxin antibodies in a murine pneumonia model.
30. Li M, Cheung GY, Hu J, et al. Comparative analysis of virulence and toxin expression of global community-associated methicillin-resistant *Staphylococcus aureus* strains. J. Infect. Dis. 2010; 202(12):1866–1876. [PubMed: 21050125]
31. Cremieux AC, Dumitrescu O, Lina G, et al. Panton–Valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. PLoS ONE. 2009; 4(9):e7204. [PubMed: 19779608]
32. Diep BA, Chan L, Tattavin P, et al. Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton–Valentine leukocidin-induced lung inflammation and injury. Proc. Natl Acad. Sci. USA. 2010; 107(12):5587–5592. [PubMed: 20231457]
33. Bae IG, Tonthat GT, Stryjewski ME, et al. Presence of genes encoding the Panton–Valentine leukocidin exotoxin is not the primary determinant of outcome in patients with complicated skin and skin structure infections due to methicillin-resistant *Staphylococcus aureus*: results of a multinational trial. J. Clin. Microbiol. 2009; 47(12):3952–3957. [PubMed: 19846653] • Large epidemiological study showing that the presence of PVL-encoding genes in the infecting

methicillin-resistant *Staphylococcus aureus* (MRSA) strain is not associated with a worse clinical outcome.

34. Yoong P, Pier GB. Antibody-mediated enhancement of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Proc. Natl Acad. Sci. USA*. 2010; 107(5):2241–2246. [PubMed: 20133867] • Shows that antibodies to PVL enhance host susceptibility to experimental skin infection in mice.
35. Wang R, Braughton KR, Kretschmer D, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat. Med.* 2007; 13(12):1510–1514. [PubMed: 17994102]
36. Kennedy AD, Bubeck Wardenburg J, Gardner DJ, et al. Targeting of α -hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. *J. Infect. Dis.* 2010; 202(7):1050–1058. [PubMed: 20726702] • Demonstrates efficient immunization based on α -toxin in a mouse model of community-acquired MRSA infection.

Website

101. *Staphylococcus aureus* Toxoids Phase 1–2 Vaccine Trial.
<http://clinicaltrials.gov/ct2/show/NCT01011335>