VIRULENCE
2017, VOL. 8, NO. 6, 607–610
https://doi.org/10.1080/21505594.2016.1255399

Taylor & Francis
Taylor & Francis Group

EDITORIAL

Superantigens of a superbug: Major culprits of *Staphylococcus aureus* disease?

M. Javad Aman

Research & Development, Integrated BioTherapeutics, Inc., Rockville, MD, USA

ARTICLE HISTORY Received 21 October 2016; Revised 21 October 2016; Accepted 24 October 2016

KEYWORDS enterotoxins; sepsis; *Staphylococcus aureus*; superantigens; toxoid vaccine; toxic shock; USA300

Infections with multidrug resistant (MDR) bacteria represent a major challenge for public health. Methicillin resistant *S. aureus* (MRSA) is one of the leading causes of hospital and community acquired infections including bacteremia and sepsis, skin and soft tissue infections, wound and implant associated infections, infective endocarditis (IE), and osteomyelitis.[1] Efforts to develop vaccine and immunotherapeutics against *S. aureus* have largely focused on surface antigens. Unfortunately, to date, all Phase II/III clinical trials have failed to demonstrate protective efficacy against *S. aureus* clinical disease. These failed attempts include vaccines based on capsular polysaccharides (StaphVax) and the iron regulated protein ISdB (V710),[2] as well as immunotherapy with an antibody against lipoteichoic acid (Pagibaximab)[3] and plasma-derived, donor-selected polyclonal immmunoglobuline enriched in antibodies to *S. aureus* adhesins (Veronate)[4] that were tested in very low birth weight infants. In fact clinical results suggests that vaccination with ISdB may have worsened the outcome of *S. aureus* infection as vaccinated individuals showed significantly higher rate of multi-organ failure.[5] A recent study also suggested a deleterious effect on subsequent *S. aureus* infection in rabbits vaccinated with a crude surface antigen preparation.[6] These findings reflect the complex nature of *S. aureus* interactions with the host and challenges facing vaccine development for this pathogen. In the face of these failures and the growing threat of antibiotic resistance exploring novel approaches for immunoprophylaxis and immunotherapy of *S. aureus* infections is imperative.

An alternative to surface antigens is a toxoid-based vaccine. Toxoids have been successful as vaccines for several pathogens such as diphtheria, tetanus, and pertussis. While mounting evidence suggests a critical role for secreted toxins in *S. aureus* pathogenesis, a major challenge for development of staphylococcal toxoid vaccines is the remarkably large number of toxins produced by this pathogen. Furthermore, various toxins are not equally important for various *S. aureus* diseases. One group of these toxins comprises of superantigens (SAgs) including more than 20 staphylococcal enteroxins (SEs), SE-like toxins (SEl), and toxic shock toxin syndrome toxin 1 (TSST-1).[7] Superantigens cross-link the T cell receptor β chain on the surface of T cells with the major histocompatibility (MHC) class II on the surface of antigen presenting cells (APC), bypassing the conventional peptide-MHC II mediated activation of T cells.[7] As a result, superantigens activate a large fraction of T lymphocytes leading to a cytokine storm that can culminate in life threatening toxic shock syndrome (TSS). SEs, but not TSST-1, also elicit emetic responses and are the primary responsible factor for *S. aureus* mediated food poisoning.[8]

While SAgs are best studied for their role in TSS and food poisoning, several lines of evidence suggest that SAgs also play a critical role in *S. aureus* disease even in the absence of classical TSS. Over the past few years several groups have reported partial protection against *S. aureus* infections in various models using vaccines or antibodies against SEB,[9] SEA,[10] TSST-1,[11] and SEC.[12] In this issue of Virulence, Aguilar *et al.*[13] report 2 monoclonal antibodies that neutralize SEK, a superantigen produced by most isolates of USA 300, the MRSA clone that is currently circulating and is responsible for most cases of *S. aureus* invasive disease in the US.[14] SEK was described in 2001 as a potent superantigen that is lethal to rabbits (Orwin I&I 2001) but little is known about the role of SEK in USA300 pathogenesis. Aguilar *et al.* showed that combination of 2 anti-SEK antibodies provides significant protection against *S. aureus* sepsis in an intravenous challenge model in mice. USA 300 is known for its high toxigenic potential, in particular due to its ability to produce panton-valentine leucocidin, α

CONTACT M. Javad Aman javad@integratedbiotherapeutics.com Research & Development, Integrated BioTherapeutics, Inc., 4 Research Court, Suite 300, Rockville, MD 20850, USA.

Comment on: Aguilar JL, et al. Monoclonal antibodies protect from Staphylococcal Enterotoxin K (SEK) induced toxic shock and sepsis by USA300 *Staphylococcus aureus*. Virulence 2017; 8(6): 741-750; PMID:27715466; https://doi.org/10.1080/21505594.2016.1231295

hemolysin and other leukotoxins,[15] but this is the first report suggesting a role for SAgs in USA 300 pathogenicity. Mice receiving anti-SEK antibodies plus vancomycin were equally protected from lethal challenge as animals receiving either agent alone but the combination groups showed less morbidity than mice treated with vancomycin alone suggesting that such antibodies may be effective as adjuvant therapy in conjunction with antibiotics. This group had previously shown that an SEB-producing MRSA strain was highly virulent in sepsis, skin infection, and a thigh muscle infection models and that an anti-SEB mAb or SEB immunization provided partial to complete protection in these models.[9] In the current paper, Aguilar *et al.* also evaluated the efficacy of the combination of an anti-SEB mAb and their anti-SEK mAb against a strain (W-132) that produces both toxins. However, the combination of the 2 mAbs failed to protect against W-132 infection. This may relate to the specific toxin profile of this particular isolate, but certainly underscores the complexity of devising a universally effective anti-toxin strategy against *S. aureus*.

The study has also its limitations as it was performed with a single isolate of USA300 and there could be variability among different clinical isolates with respect to the relative role of SEK in pathogenesis. This notion is reinforced by fact that a combination of anti-SEK and anti-SEB was not protective against W-132. This is not surprising as *S. aureus* produces a plethora of toxins and the profile and expression levels of different toxins could be quite variable among different isolates as previously reported by the same team.[16,17] It remains to be seen how reproducible these findings are in a larger set of isolates, but in all likelihood, immunotherapy targeting only the superantigens will not provide broad protection. Nonetheless these data indicate that superantigens should be evaluated as potential targets in a multivalent vaccine or antibody treatment.

A number of additional studies published in the past few years point to the importance of SAgs as therapeutic or vaccine targets. Asensi *et al.* showed protection against intraperitoneal *S. aureus* challenge in mice immunized with lactobacillus expressed SEB toxoid[18] and Hu et al demonstrated protection against sepsis with a TSST-1 producing strain using an attenuated TSST-1 vaccine.[11] TSST-1 vaccination not only provided significant protection against mortality but also reduced bacterial burden in organs after intravenous challenge.[11] This group also showed protection against sepsis by an SEC-producing strain using an attenuated SEC toxoid.[19] Studies in an HLA-DR transgenic mice that are more sensitive to SAgs showed that intranasal exposure to SAgs induces airway inflammation suggesting a role for SAgs in *S. aureus* pneumonia.[20] Spaulding *et al.* reported significant protection against *S. aureus* pneumonia in rabbits using a multivalent immunization with various combinations of SAgs and cytolysins.[6] The latter study was rather surprising as vaccination with TSST-1 alone was sufficient for complete protection against USA300 LAC strain, while both α hemolysin and PVL are known to play a very critical role in protection against USA300 in the same model.[21] Nonetheless, the collective data suggest a possible role for SAgs in *S. aureus* pneumonia. In addition, a role for SAgs has been proposed in a rabbit model of *S. aureus* induced endocarditis. Using isogenic knockouts of the MRSA strain MW2, Salgado-Pabón *et al.* showed that in this strain the formation of vegetative foci in the aortic valves as well as seeding in kidney was dependent on SEC.[22] In another study, treatment with soluble, high-affinity Vβ T cell receptor chains specific for SEC reduced the vegetation and bacterial counts in rabbit model of IE.[12] Furthermore, an epidemiological study showed high prevalence of SAgs in isolates from IE cases when compared with isolates recovered from skin infections.[23]

The mechanism by which SAgs influence the course of various *S. aureus* diseases in the absence of TSS remains enigmatic. Low levels of SAgs can trigger a strong inflammatory response at the site of infection. This effect of SAgs may also be independent of T cell/APC cross linking. It has been shown that human aortic endothelial cells (HAECs) produce IL-8 in response to SEC which can recruit polymorphonuclear cells and induce tissue toxicity.[22] Other *S. aureus* toxins such as bicomponent leukocidins have also been shown to induce inflammatory processes.[24] It is possible that neutralization of these toxins modulates the local inflammatory response and this fine-tuning may be important for effective clearance of infection by neutrophils. In other words, this modulation may turn a panic attack into a measured response.

It is widely assumed that the toxoid vaccines work only by inducing neutralizing antibodies that protect the host from toxic effects of the toxin. However, toxoids as proteins can also elicit T cell responses. A recent report indicates that vaccination with non-toxic mutant TSST-1 induces IL-17-dependent protection against *S. aureus* infection.[25] IL-17A producing cells were increased in the spleen cells of mTSST-1 vaccinated mice and the partial protection against *S. aureus* observed upon mTSST-1 vaccination (strain 834) was abolished in IL-17 knockout mice.[25] In contrast, serum transfer form mTSST-1 immunized mice to naïve mice failed to protect against subsequent infection. Given these data and the critical importance of Th-17 responses in protection against *S. aureus*, potential involvement of this mechanism in protection mediated by toxoids merits further investigation. The protective effect of toxoids may also result from a combination of antibody and T cell responses where neutralizing antibodies tone down the inflammatory

response early on during the infection allowing an effective subsequent Th17 response to clear the infection.

In summary, recent findings strongly support a role for superantigens in various *S. aureus* syndromes and suggest the possibility that these toxins can be valuable targets for vaccines and immunotherapy. Recent Phase I clinical trials of recombinant toxoids for SEB (STEBVax)[26] and TSST-1[27] were important milestones in this direction as they established the safety of vaccines in humans for these 2 potent and potentially lethal toxins. However, targeting any single toxin is not sufficient for effective protection against this formidable pathogen. A viable vaccine approach must include multiple critical toxoids, possibly including superantigens. If the mechanism of action is primarily based on antibody mediated neutralization, a major challenge will be to identify a few SAg toxoids that can induce relatively broad neutralizing response. Further challenge is that the role and significance of any single toxin in different *S. aureus* syndromes could be different and it is possible that different vaccine formulation would be needed to prevent different *S. aureus* diseases.

Disclosure of potential conflicts of interest

MJA has stocks in Integrated Biotherapeutics, Inc.

References

[1] Lowy FD. Staphylococcus aureus infections. N Engl J Med 1998; 339:520-32; PMID:9709046; https://doi.org/10.1056/NEJM199808203390806

[2] Fowler VG, Jr, Proctor RA. Where does a Staphylococcus aureus vaccine stand? Clin Microbiol Infect 2014; 20 Suppl 5:66-75; PMID:24476315; https://doi.org/10.1111/1469-0691.12570

[3] Patel M, Kaufman DA. Anti-lipoteichoic acid monoclonal antibody (pagibaximab) studies for the prevention of staphylococcal bloodstream infections in preterm infants. Expert Opin Biol Ther 2015; 15:595-600; PMID:25736524; https://doi.org/10.1517/14712598.2015.1019857

[4] DeJonge M, Burchfield D, Bloom B, Duenas M, Walker W, Polak M, Jung E, Millard D, Schelonka R, Eyal F, et al. Clinical trial of safety and efficacy of INH-A21 for the prevention of nosocomial staphylococcal bloodstream infection in premature infants. J Pediatr 2007; 151:260-5; PMID:17719934; https://doi.org/10.1016/j.jpeds.2007.04.060

[5] Fowler VG, Allen KB, Moreira ED, Moustafa M, Isgro F, Boucher HW, Corey GR, Carmeli Y, Betts R, Hartzel JS, et al. Effect of an investigational vaccine for preventing Staphylococcus aureus infections after cardiothoracic surgery: a randomized trial. JAMA 2013; 309:1368-78; PMID:23549582; https://doi.org/10.1001/jama.2013.3010

[6] Spaulding AR, Salgado-Pabon W, Merriman JA, Stach CS, Ji Y, Gillman AN, Peterson ML, Schlievert PM. Vaccination Against Staphylococcus aureus Pneumonia. J Infect Dis 2014; 209:1955-62

[7] Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. Immunol Rev 2008; 225:226-43; PMID:18837785; https://doi.org/10.1111/j.1600-065X.2008.00681.x

[8] Hu DL, Nakane A. Mechanisms of staphylococcal enterotoxin-induced emesis. Eur J Pharmacol 2014; 722:95-107; PMID:24184671; https://doi.org/10.1016/j.ejphar.2013.08.050

[9] Varshney AK, Wang X, Scharff MD, Macintyre J, Zollner RS, Kovalenko OV, Martinez LR, Byrne FR, Fries BC. Staphylococcal Enterotoxin B-Specific Monoclonal Antibody 20B1 Successfully Treats Diverse Staphylococcus aureus Infections. J Infect Dis 2013; 208:2058-66; PMID:23922375

[10] Nilsson IM, Lee JC, Bremell T, Ryden C, Tarkowski A. The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis. Infect Immun 1997; 65:4216-21; PMID:9317029.

[11] Hu DL, Omoe K, Sasaki S, Sashinami H, Sakuraba H, Yokomizo Y, Shinagawa K, Nakane A. Vaccination with nontoxic mutant toxic shock syndrome toxin 1 protects against Staphylococcus aureus infection. J Infect Dis 2003; 188:743-52; PMID:12934191; https://doi.org/10.1086/377308

[12] Mattis DM, Spaulding AR, Chuang-Smith ON, Sundberg EJ, Schlievert PM, Kranz DM. Engineering a soluble high-affinity receptor domain that neutralizes staphylococcal enterotoxin C in rabbit models of disease. Protein Eng Des Sel 2013; 26:133-42; PMID:23161916; https://doi.org/10.1093/protein/gzs094

[13] Aguilar JL, Varshney AK, Pechuan X, Dutta K, Nosanchuk JD, Fries BC. Monoclonal antibodies protect from Staphylococcal Enterotoxin K (SEK) induced toxic shock and sepsis by USA300 *Staphylococcus aureus*. Virulence 2017; 8(6): 741-750; PMID:27715466; https://doi.org/10.1080/21505594.2016.1231295

[14] David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010; 23:616-87; PMID:20610826; https://doi.org/10.1128/CMR.00081-09

[15] Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003; 9:978-84; PMID:12967497; https://doi.org/10.3201/eid0908.030089

[16] Aguilar JL, Varshney AK, Wang X, Stanford L, Scharff M, Fries BC. Detection and measurement of staphylococcal enterotoxin-like K (SEl-K) secretion by Staphylococcus aureus clinical isolates. J Clin Microbiol 2014; 52:2536-43; PMID:24808237; https://doi.org/10.1128/JCM.00387-14

[17] Varshney AK, Mediavilla JR, Robiou N, Guh A, Wang X, Gialanella P, Levi MH, Kreiswirth BN, Fries BC. Diverse enterotoxin gene profiles among clonal complexes of Staphylococcus aureus isolates from the Bronx, New York. Appl Environ Microbiol 2009; 75:6839-49; PMID:19749060; https://doi.org/10.1128/AEM.00272-09

[18] Asensi GF, de Sales NF, Dutra FF, Feijo DF, Bozza MT, Ulrich RG, Miyoshi A, de Morais K, Azevedo VA, Silva JT, et al. Oral immunization with Lactococcus lactis secreting attenuated recombinant staphylococcal enterotoxin B induces a protective immune response in a murine model. Microb Cell Fact 2013; 12:32; PMID:23561053; https://doi.org/10.1186/1475-2859-12-32

[19] Hu DL, Omoe K, Narita K, Cui JC, Shinagawa K, Nakane A. Intranasal vaccination with a double mutant of staphylococcal enterotoxin C provides protection against Staphylococcus aureus infection. Microbes Infect 2006; 8:2841-8; PMID:17090392; https://doi.org/10.1016/j.micinf.2006.09.001

[20] Rajagopalan G, Sen MM, Singh M, Murali NS, Nath KA, Iijima K, Kita H, Leontovich AA, Gopinathan U, Patel R, et al. Intranasal exposure to staphylococcal enterotoxin B elicits an acute systemic inflammatory response. Shock 2006; 25:647-56; PMID:16721274; https://doi.org/10.1097/01.shk.0000209565.92445.7d

[21] Diep BA, Le VT, Visram ZC, Rouha H, Stulik L, Dip EC, Nagy G, Nagy E. Improved Protection in a Rabbit Model of Community-Associated Methicillin-Resistant Staphylococcus aureus Necrotizing Pneumonia upon Neutralization of Leukocidins in Addition to Alpha-Hemolysin. Antimicrob Agents Chemother 2016; 60:6333-40; PMID:27527081; https://doi.org/10.1128/AAC.01213-16

[22] Salgado-Pabon W, Breshears L, Spaulding AR, Merriman JA, Stach CS, Horswill AR, Peterson ML, Schlievert PM. Superantigens are critical for Staphylococcus aureus Infective endocarditis, sepsis, and acute kidney injury. MBio 2013; 4:e00494-13; PMID:23963178; https://doi.org/10.1128/mBio.00494-13

[23] Nienaber JJ, Sharma Kuinkel BK, Clarke-Pearson M, Lamlertthon S, Park L, Rude TH, Barriere S, Woods CW, Chu VH, Marín M, et al. Methicillin-susceptible Staphylococcus aureus endocarditis isolates are associated with clonal complex 30 genotype and a distinct repertoire of enterotoxins and adhesins. J Infect Dis 2011; 204:704-13; PMID:21844296; https://doi.org/10.1093/infdis/jir389

[24] Vandenesch F, Lina G, Henry T. Staphylococcus aureus hemolysins, bi-component leukocidins, and cytolytic peptides: a redundant arsenal of membrane-damaging virulence factors? Front Cell Infect Microbiol 2012; 2:12; PMID:22919604; https://doi.org/10.3389/fcimb.2012.00012

[25] Narita K, Hu DL, Asano K, Nakane A. Vaccination with non-toxic mutant toxic shock syndrome toxin-1 induces IL-17-dependent protection against Staphylococcus aureus infection. Pathog Dis 2015; 73: 00399-16; PMID:25857736; https://doi.org/10.1093/femspd/ftv023

[26] Chen WH, Pasetti MF, Adhikari RP, Baughman H, Douglas R, El-Khorazaty J, Greenberg N, Holtsberg FW, Liao GC, Reymann MK, et al. The safety and immunogenicity of a parenterally administered structure-based rationally modified recombinant Staphylococcal enterotoxin B protein vaccine, STEBVax. Clin Vaccine Immunol 2016; 6(9); 1036-44

[27] Schwameis M, Roppenser B, Firbas C, Gruener CS, Model N, Stich N, Roetzer A, Buchtele N, Jilma B, Eibl MM. Safety, tolerability, and immunogenicity of a recombinant toxic shock syndrome toxin (rTSST)-1 variant vaccine: a randomised, double-blind, adjuvant-controlled, dose escalation first-in-man trial. Lancet Infect Dis 2016; 16:1036-44; PMID:27296693; https://doi.org/10.1016/S1473-3099(16)30115-3