Published in final edited form as:
J Mol Med (Berl). 2010 February ; 88(2): 109–114. doi:10.1007/s00109-009-0573-x.

Community-associated methicillin-resistant *Staphylococcus aureus* immune evasion and virulence

Shawna F. Graves, **Scott D. Kobayashi**, and **Frank R. DeLeo**
Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840, USA, fdeleo@niaid.nih.gov

Abstract

Staphylococcus aureus is a significant cause of human infections globally. Methicillin-resistant *S. aureus* (MRSA) emerged in the early 1960s and is now endemic in most healthcare facilities. Although healthcare-associated MRSA infections remain a major problem in most industrialized countries, those caused by community-associated MRSA (CA-MRSA) are now the most abundant cause of bacterial infections in the community in some parts of the world, such as the United States. The basis for the emergence and subsequent success of CA-MRSA is incompletely defined. However, the ability of the pathogen to cause disease in otherwise healthy individuals is likely attributed, in part, to its ability to circumvent killing by the innate immune system, which includes survival after phagocytosis by neutrophils. In this review, we discuss the role of neutrophils in host defense against *S. aureus* and highlight progress made toward understanding mechanisms of CA-MRSA virulence and pathogenesis.

Keywords

Neutrophil; Virulence; Host defense; *Staphylococcus aureus*; CA-MRSA; Innate immunity; Infection

Introduction

Staphylococcus aureus is a leading cause of bacterial infections worldwide. The pathogen has capacity to cause a wide range of syndromes of varied severity. For example, *S. aureus* is the most abundant cause of skin and soft tissue infections in most industrialized countries, including the United States [1]. Such infections are often relatively minor. On the other hand, *S. aureus* is a leading cause of bloodstream infections in the healthcare setting, many of which are fatal [2]. Given the high prevalence of *S. aureus* infections, it is, perhaps, not surprising that ~30% of non-institutionalized individuals are permanently colonized with the organism [3,4]. Therefore, *S. aureus* may be considered a component of normal human flora.

S. aureus is widely known for its ability to rapidly acquire resistance to antibiotics. For instance, although penicillin was initially highly effective in the treatment of staphylococcal infections [5], by the late 1940s, penicillin resistance had increased such that the antibiotic was no longer an effective agent for treatment of *S. aureus* infections [6]. Similarly, methicillin-resistant *S. aureus* (MRSA) were reported 2 years after methicillin was

Correspondence to: Frank R. DeLeo.

Conflict of interest statement The authors declare no conflict of interest.

introduced for treatment of penicillin-resistant *S. aureus* infections [7]. MRSA has since spread worldwide and is now endemic in hospitals and healthcare settings of most industrialized countries (reviewed in [8]). Notably, MRSA may be the leading cause of mortality by any single infectious agent in the United States ([2] and Fig. 1 in [9]).

Community-associated MRSA (CA-MRSA) infections were first reported in indigenous populations of Western Australia in the early 1990s [10] and subsequently among children in the Midwestern region of the United States [11,12]. Unexpectedly, CA-MRSA disseminated rapidly and is now epidemic in the United States—it is the most abundant cause of bacterial infections in the community [13]. In contrast to healthcare-associated MRSA (HA-MRSA) infections, for which there are predisposing risk factors or underlying susceptibilities, CA-MRSA infections occur in otherwise healthy individuals with no exposure to the healthcare setting. Thus, anyone is at risk for infection. These observations imply that CA-MRSA strains have enhanced virulence compared with traditional HA-MRSA strains, and recent studies have addressed this notion in animal infection models or experiments with human leukocytes in vitro (see below). Although the vast majority of CA-MRSA infections are of skin and soft tissue, the pathogen has capacity to cause severe invasive disease [14,15]. Direct contact with an infected or colonized individual is likely the primary means of transmission (see http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_clinicians.html#8).

CA-MRSA strains have a unique methicillin resistance element, known as staphylococcal cassette chromosome *mec* type IV or V (SCC*mec*IV or V), whereas traditional HA-MRSA strains contain a larger SCC*mec* element (types I–III) ([16] and reviewed in [8]). Initially, the presence of genes encoding Panton–Valentine leukocidin (PVL, encoded by *lukS-PV* and *lukF-PV*), a two-component cytolytic toxin, and SCC*mec*IV was associated with emergent CA-MRSA strains worldwide [17]. Although all CA-MRSA strains contain SCC*mec*IV or V, recent reports indicate some CA-MRSA strains lack genes encoding PVL [18–21]. The molecular basis for enhanced virulence and transmission of CA-MRSA, which includes understanding interaction of this pathogen with the innate immune system, remains an intense area of investigation.

Here, we review our current knowledge of host defense against *S. aureus*, with emphasis on the enhanced ability of CA-MRSA to circumvent killing by neutrophils, and provide an update on CA-MRSA virulence mechanisms.

Neutrophils and bacterial immune evasion

Neutrophils or polymorphonuclear leukocytes (PMNs) are the most abundant of all leukocytes and are essential for host defense against invading pathogenic microbes. The importance of PMNs in host defense against bacterial pathogens is exemplified by pronounced susceptibility to infection in patients with primary neutrophil defects. For example, individuals with severe congenital neutropenia, leukocyte adhesion deficiency, chronic granulomatous disease, and neutrophil-specific granule deficiency are at increased risk for developing recurrent *S. aureus* infections [22]. PMNs are terminally differentiated innate immune cells and following maturation in bone marrow are released into circulation. Neutrophils are rapidly recruited to infected tissue through host chemotactic signals delivered by cytokines and chemokines, as well as bacteria-derived products such as *N*-formyl peptides. PMNs recognize and ingest bacterial pathogens at sites of infection though a process known as phagocytosis, which is facilitated by opsonization of the microbial surface with host serum proteins such as antibody and complement. Phagocytosis of bacteria induces production of microbicidal reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, and chloramines [23]. In addition to ROS,

bacteria are exposed to antimicrobial peptides and proteases following fusion of cytoplasmic granules with pathogen-containing phagosomes [24]. Together, oxygen-dependent and oxygen-independent neutrophil killing mechanisms are sufficient to kill most invading microorganisms. Notwithstanding, bacterial pathogens such as *S. aureus* and CA-MRSA, in particular, survive following neutrophil phagocytosis and thereby cause disease [25–27]. Inasmuch as PMNs are essential to host defense, *S. aureus* evasion of the innate immune system represents an important mechanism for pathogenesis. Some of the specific immune evasion strategies employed by *S. aureus* are considered below.

Resolution of infection

Influx to and accumulation of neutrophils at sites of infection is a critical component of the acute inflammatory response and required for resolution of bacterial disease. Notwithstanding, the degradative enzymes and cytotoxic molecules that contribute to PMN bactericidal activity are potentially harmful to host tissue should the neutrophil lyse. For instance, neutrophils are intimately associated with host inflammatory disorders such as rheumatoid arthritis. Thus, circumspect removal of activated PMNs from infection sites is paramount to the resolution of inflammation [28]. Neutrophils undergo either accelerated apoptosis following phagocytosis to resolve the inflammatory process or a slower spontaneous apoptosis to maintain immune system homeostasis. In either scenario, macrophages are essential to recognize and remove effete PMNs through a process called efferocytosis [28]. Several bacterial pathogens alter this conventional innate immune process as a mechanism for pathogenesis (Fig. 1). For example, the pathogen *Anaplasma phagocytophilum* inhibits neutrophil apoptosis to facilitate an obligate intracellular lifecycle. By contrast, pathogens such as *S. aureus* promote neutrophil lysis and thereby survive and cause disease. Although most *S. aureus* strains eventually cause PMN lysis, it is noteworthy that prominent CA-MRSA strains are particularly adept at inducing rapid neutrophil lysis (Fig. 1) [26]. Thus, the ability of prominent USA300 and USA400 to cause rapid PMN lysis may contribute to increased virulence of these strains.

CA-MRSA virulence and immune evasion

S. aureus produces numerous surface molecules and freely secreted toxins that have potential to alter or inhibit function of innate immune cells (reviewed in [29]). In addition to the large repertoire of putative *S. aureus* immune evasion factors, the significant redundancy of function among many of these factors suggests that no single molecule can uniquely explain the ability of *S. aureus* to cause human disease. For example, *S. aureus* has capacity to generate multiple molecules that inhibit neutrophil chemotaxis and complement function, sequester host antibody, promote resistance to antimicrobial peptides or reactive oxygen species, and cause host cell lysis [29]. Here, we focus our discussion on the processes and molecules implicated in the enhanced virulence phenotype of CA-MRSA.

CA-MRSA strains are highly virulent in mouse infection models

S. aureus stains known as USA300 [30,31] and USA400 [32] are the most abundant cause of CA-MRSA infections in the United States, although the number of USA300 infections now far exceeds those caused by USA400 [13,33,34]. Voyich et al. were the first to compare virulence of USA300 (strain LAC) and USA400 (strains MW2 and MnCop) with early or representative HA-MRSA strains, COL, and MRSA252 [26]. The authors found that CA-*S. aureus* strains, which included CA-MRSA and CA-MSSA (MnCop) strains, were significantly more virulent than either COL or MRSA252 in a mouse sepsis model, and follow-up studies with an extended time course confirmed the original findings [35]. Although neutrophil phagocytosis and activation were similar among all strains tested in vitro, survival of USA300 and USA400 was significantly greater after phagocytosis

compared with COL or MRSA252. The molecular basis of enhanced survival of these CA-MRSA strains after phagocytosis is currently under investigation, but, as noted above, it ultimately results in destruction of neutrophils. In addition, the observation that MW2 and MnCop have comparable virulence in animal models suggests that methicillin resistance per se has no impact on virulence. This idea was confirmed in subsequent studies by Diep et al. [36].

Molecules implicated in CA-MRSA virulence

Inasmuch as the genes encoding PVL are present with many CA-MRSA strains, it has been suggested over the past several years that the toxin might be responsible for the emergence, transmission, and/or enhanced virulence of CA-MRSA. This notion seems logical, since PVL is a known virulence factor of *S. aureus* and unique among the earliest CA-MRSA strains. In addition, a previous pandemic of community-associated infections was caused by a PVL-positive strain known as phage type 80/81 [37]. As a first step toward testing this notion, Voyich et al. constructed USA300 and USA400 isogenic *lukS-PV* and *lukF-PV*-negative strains (Δ*pvl*) and compared virulence with the parental wild-type strains in mouse skin infection and sepsis models [35]. Unexpectedly, the wild-type and isogenic Δ*pvl* strains had comparable ability to cause disease in these mouse models of CA-MRSA infection. In addition, survival of these wild-type and Δ*pvl* strains following phagocytosis by human neutrophils was comparable [35]. Bubeck Wardenburg et al. were the first to evaluate wild-type and isogenic Δ*pvl* CA-MRSA strains in a mouse pneumonia model and found comparable mortality between wild-type and Δ*pvl* strains at all time points tested [38]. Since the first studies with USA300 and USA400 strains were published, there have been several other reports investigating the role of PVL in virulence using mouse, rat, or rabbit infection models [39–44]. Conclusions from the majority of studies with rodent models concur [35,38,39,41,43] or variance can be explained by the model used [39] or an unintended genetic mutation [44,45], with one exception [40]. The work by Brown et al. [40] used USA300 strains and animal infection models that were either identical or virtually identical to those of Bubeck Wardenburg et al. [43], so, the basis for the differences in experimental outcome is not clear. It is important to note that PVL is epidemiologically linked to certain types of disease, such as severe necrotizing pneumonia and some types of severe skin infection [46,47]. Therefore, the findings in animal models published to date may simply indicate that PVL has little or no significant role in most CA-MRSA infections but can contribute to some types of severe infection, which are typically infrequent and may involve an underlying human susceptibility factor. That said, one must consider the limitations of animal models in general for predicting the role of virulence factors in human infection, as there is increasing evidence for the host-specific activity of staphylococcal virulence factors [48–50].

There has been concerted effort over the past few years to determine the molecular basis of enhanced virulence and transmission of USA300. During the course of such work, Wang et al. identified novel *S. aureus* peptides with limited homology to the phenol-soluble modulins (PSMs) of *Staphylococcus epidermidis* [51]. A subset of these peptides, known as α-type PSMs (PSMα), is ~20 amino acids and recruits and lyses neutrophils. In accordance with these in vitro data, USA300 and USA400 isogenic Δ*psmα* deletion strains have significantly reduced virulence in mouse sepsis and skin infection models, indicating PSMα peptides are major determinants of CA-MRSA virulence [51]. It is also notable that PSMα peptides are produced in vitro at significantly higher concentrations by USA300 compared with the most prominent HA-MRSA pulsed-field types, USA100 and USA200 [52]. The relatively high expression of PSMα peptides by USA300 is, in part, related to the high activity of accessory gene regulator (*agr*) in this strain [52]. An intriguing idea proposed by Li et al. [52] is that the enhanced virulence phenotype of USA300 is based largely on differential expression of

core-genome encoded virulence factors such as PSMα peptides and alpha-hemolysin (alpha-toxin, Hla) rather than acquisition of mobile genetic elements [52].

Recent work by Bubeck Wardenburg et al. revealed that Hla plays prominent role in pathogenesis of *S. aureus* pneumonia [38,53,54]. An isogenic *hla*-deficient strain of USA300 was essentially avirulent in the mouse pneumonia [38,43] and active or passive immunization against Hla protected animals from death [53]. Although this cytolytic toxin has long been known to be a virulence factor of *S. aureus*, these studies provide strong support to the idea that Hla is one of the primary determinants of (if not the major determinant) virulence in *S. aureus* pneumonia. These findings are underscored by recent observations that indicate expression of Hla by USA300 is relatively high compared with other strains of the same clonal complex [52]. Work by Bartlett et al. investigated the mechanism of action of Hla in vivo and found that the toxin elicits host CXC chemokine production in a mouse *S. aureus* pneumonia model [55]. These chemokines promote influx of neutrophils, which, in turn, may be largely responsible for lung injury during infection. Such a process would be consistent with the host–pathogen model described above, whereby lysis of neutrophils after phagocytosis of *S. aureus* could contribute to host tissue destruction.

Concluding remarks

S. aureus continues to be a leading cause of bacterial infections in healthcare settings worldwide. However, the recent emergence of highly virulent CA-MRSA strains has changed the epidemiology of severe *S. aureus* infections such that all populations can be considered at risk. As discussed in this review, it is increasingly clear that prominent CA-MRSA strains are particularly adept at evasion of the innate immune response—a feature that likely contributes to enhanced virulence. Although progress has been made towards identifying factors involved in CA-MRSA virulence, several key questions remain unanswered. For example, the molecular basis of enhanced USA300 transmission remains incompletely determined. Understanding the enhanced transmission phenotype will likely provide new insight into the basis for emergence of epidemic *S. aureus* clones. Also, our knowledge of the host genetics of susceptibility to severe *S. aureus* infections is limited and must be addressed. A complete understanding of molecules that impact evolution of epidemic CA-MRSA strains will be essential to guide development of novel therapeutics for prevention and treatment of severe *S. aureus* disease.

Acknowledgments

This article was supported by the Intramural Research Program of the National Institutes of Allergy and Infectious Diseases, National Institutes of Health. The authors thank Anita Mora, NIAID, for help in generating the figure.

References

1. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin Infect Dis. 2001; 32(Suppl 2):S114–S132. [PubMed: 11320452]

2. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA. 2007; 298:1763–1771. [PubMed: 17940231]

3. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, Jensen BJ, Killgore G, Tenover FC, Kuehnert MJ. Changes in the prevalence of nasal colonization with

Staphylococcus aureus in the United States, 2001–2004. J Infect Dis. 2008; 197:1226–1234. [PubMed: 18422434]

4. Graham PL III, Lin SX, Larson EL. A U.S. population-based survey of *Staphylococcus aureus* colonization. Ann Intern Med. 2006; 144:318–325. [PubMed: 16520472]
5. Spink WW, Hall WH, Ferris V. Clinical significance of staphylococci with natural or acquired resistance to the sulfonamides and to penicillin. JAMA. 1945; 128:555–559.
6. Barber M, Rozwadowska-Dowzenko M. Infection by penicillin-resistant staphylococci. Lancet. 1948; 252:641–644. [PubMed: 18890505]
7. Jevons MP. "Celbenin"-resistant staphylococci. Br Med J. 1961; 1:124–125.
8. Chambers HF, DeLeo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol. 2009; 7:2464–2474.
9. DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. J Clin Invest. 2009; 119:2464–2474. [PubMed: 19729844]
10. Udo EE, Pearman JW, Grubb WB. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J Hosp Infect. 1993; 25:97–108. [PubMed: 7903093]
11. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, Leitch CD, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA. 1998; 279:593–598. [PubMed: 9486753]
12. Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. JAMA. 1999; 282:1123–1125. [PubMed: 10501104]
13. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med. 2006; 355:666–674. [PubMed: 16914702]
14. Adem PV, Montgomery CP, Husain AN, Koogler TK, Arangelovich V, Humilier M, Boyle-Vavra S, Daum RS. *Staphylococcus aureus* sepsis and the Waterhouse–Friderichsen syndrome in children. N Engl J Med. 2005; 353:1245–1251. [PubMed: 16177250]
15. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med. 2005; 352:1445–1453. [PubMed: 15814880]
16. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, Coombs GW, Pearman JW, Tenover FC, Kapi M, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol. 2002; 40:4289–4294. [PubMed: 12409412]
17. 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; 9:978–984. [PubMed: 12967497]
18. Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton–Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. J Clin Microbiol. 2007; 45:2554–2563. [PubMed: 17581935]
19. Zhang K, McClure JA, Elsayed S, Tan J, Conly JM. Coexistence of Panton–Valentine leukocidin-positive and leukocidin-negative community-associated methicillin-resistant *Staphylococcus aureus* USA400 sibling strains in a large Canadian health-care region. J Infect Dis. 2008; 197:195–204. [PubMed: 18173361]
20. O'Brien FG, Lim TT, Chong FN, Coombs GW, Enright MC, Robinson DA, Monk A, Said-Salim B, Kreiswirth BN, Grubb WB. Diversity among community isolates of methicillin-resistant *Staphylococcus aureus* in Australia. J Clin Microbiol. 2004; 42:3185–3190. [PubMed: 15243080]
21. Kim ES, Song JS, Lee HJ, Choe PG, Park KH, Cho JH, Park WB, Kim SH, Bang JH, Kim DM, et al. A survey of community-associated methicillin-resistant *Staphylococcus aureus* in Korea. J Antimicrob Chemother. 2007; 60:1108–1114. [PubMed: 17884831]

22. Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in phagocytes. N Engl J Med. 2000; 343:1703–1714. [PubMed: 11106721]
23. Rosen H, Klebanoff SJ. Bactericidal activity of a superoxide anion-generating system. A model for the polymorphonuclear leukocyte. J Exp Med. 1979; 149:27–39. [PubMed: 216766]
24. Cohn ZA, Hirsch JG. The influence of phagocytosis on the intracellular distribution of granule-associated components of polymorphonuclear leucocytes. J Exp Med. 1960; 112:1015–1022. [PubMed: 13694489]
25. Rogers DE, Tompsett R. The survival of staphylococci within human leukocytes. J Exp Med. 1952; 95:209–230. [PubMed: 14907971]
26. Voyich JM, Braughton KR, Sturdevant DE, Whitney AR, Said-Salim B, Porcella SF, Long RD, Dorward DW, Gardner DJ, Kreiswirth BN, et al. Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. J Immunol. 2005; 175:3907–3919. [PubMed: 16148137]
27. Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindberg FP. Survival of *Staphylococcus aureus* inside neutrophils contributes to infection. J Immunol. 2000; 164:3713–3722. [PubMed: 10725730]
28. Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM, Haslett C. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest. 1989; 83:865–875. [PubMed: 2921324]
29. DeLeo FR, Diep BA, Otto M. Host defense and pathogenesis in *Staphylococcus aureus* infections. Infect Dis Clin North Am. 2009; 23:17–34. [PubMed: 19135914]
30. Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet. 2006; 367:731–739. [PubMed: 16517273]
31. Kennedy AD, Otto M, Braughton KR, Whitney AR, Chen L, Mathema B, Mediavilla JR, Byrne KA, Parkins LD, Tenover FC, et al. Epidemic community-associated methicillin-resistant *Staphylococcus aureus*: recent clonal expansion and diversification. Proc Natl Acad Sci USA. 2008; 105:1327–1332. [PubMed: 18216255]
32. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, Nagai Y, Iwama N, Asano K, Naimi T, et al. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet. 2002; 359:1819–1827. [PubMed: 12044378]
33. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. Ann Intern Med. 2006; 144:309–317. [PubMed: 16520471]
34. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol. 2003; 41:5113–5120. [PubMed: 14605147]
35. Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, Long RD, Dorward DW, Gardner DJ, Lina G, et al. Is Panton–Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? J Infect Dis. 2006; 194:1761–1770. [PubMed: 17109350]
36. Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, Jones A, Pallazolo-Ballance AM, Perdreau-Remington F, Sensabaugh GF, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. J Infect Dis. 2008; 197:1523–1530. [PubMed: 18700257]
37. Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, et al. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired meticillin-resistant clone. Lancet. 2005; 365:1256–1258. [PubMed: 15811459]

38. Bubeck Wardenburg J, Bae T, Otto M, DeLeo FR, Schneewind O. Poring over pores: alpha-hemolysin and Panton–Valentine leukocidin in *Staphylococcus aureus* pneumonia. Nat Med. 2007; 13:1405–1406. [PubMed: 18064027]
39. Tseng CW, Kyme P, Low J, Rocha MA, Alsabeh R, Miller LG, Otto M, Arditi M, Diep BA, Nizet V, et al. *Staphylococcus aureus* Panton–Valentine leukocidin contributes to inflammation and muscle tissue injury. PLoS ONE. 2009; 4:e6387. [PubMed: 19633710]
40. Brown EL, Dumitrescu O, Thomas D, Badiou C, Koers EM, Choudhury P, Vazquez V, Etienne J, Lina G, Vandenesch F, et al. The Panton–Valentine leukocidin vaccine protects mice against lung and skin infections caused by *Staphylococcus aureus* USA300. Clin Microbiol Infect. 2009; 15:156–164. [PubMed: 19154186]
41. 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; 77:2159–2167. [PubMed: 19237525]
42. Diep BA, Palazzolo-Ballance AM, Tattevin P, Basuino L, Braughton KR, Whitney AR, Chen L, Kreiswirth BN, Otto M, DeLeo FR, et al. Contribution of Panton–Valentine leukocidin in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. PLoS ONE. 2008; 3:e3198. [PubMed: 18787708]
43. 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; 198:1166–1170. [PubMed: 18729780]
44. Labandeira-Rey M, Couzon F, Boisset S, Brown EL, Bes M, Benito Y, Barbu EM, Vazquez V, Hook M, Etienne J, et al. *Staphylococcus aureus* Panton–Valentine leukocidin causes necrotizing pneumonia. Science. 2007; 315:1130–1133. [PubMed: 17234914]
45. Villaruz AE, Bubeck Wardenburg J, Khan BA, Whitney AR, Sturdevant DE, Gardner DJ, DeLeo FR, Otto M. 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; 200:724–734. [PubMed: 19604047]
46. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. Involvement of Panton–Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis. 1999; 29:1128–1132. [PubMed: 10524952]
47. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piemont Y, Brousse N, Floret D, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton–Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet. 2002; 359:753–759. [PubMed: 11888586]
48. Hongo I, Baba T, Oishi K, Morimoto Y, Ito T, Hiramatsu K. Phenol-soluble modulin alpha 3 enhances the human neutrophil lysis mediated by Panton–Valentine leukocidin. J Infect Dis. 2009; 200:715–723. [PubMed: 19653829]
49. de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier MJ, Van Kessel KP, Van Strijp JA. Chemotaxis inhibitory protein of *Staphylococcus aureus*, a bacterial antiinflammatory agent. J Exp Med. 2004; 199:687–695. [PubMed: 14993252]
50. Szmigielski S, Prevost G, Monteil H, Colin DA, Jeljaszewicz J. Leukocidal toxins of staphylococci. Zentralbl Bakteriol. 1999; 289:185–201. [PubMed: 10360319]
51. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med. 2007; 13:1510–1514. [PubMed: 17994102]
52. Li M, Diep BA, Villaruz AE, Braughton KR, Jiang X, DeLeo FR, Chambers HF, Lu Y, Otto M. Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci U S A. 2009; 106:5883–5888. [PubMed: 19293374]
53. Bubeck Wardenburg J, Schneewind O. Vaccine protection against *Staphylococcus aureus* pneumonia. J Exp Med. 2008; 205:287–294. [PubMed: 18268041]

54. Bubeck WJ, Patel RJ, Schneewind O. Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. Infect Immun. 2007; 75:1040–1044. [PubMed: 17101657]

55. Bartlett AH, Foster TJ, Hayashida A, Park PW. Alpha-toxin facilitates the generation of CXC chemokine gradients and stimulates neutrophil homing in *Staphylococcus aureus* pneumonia. J Infect Dis. 2008; 198:1529–1535. [PubMed: 18823272]

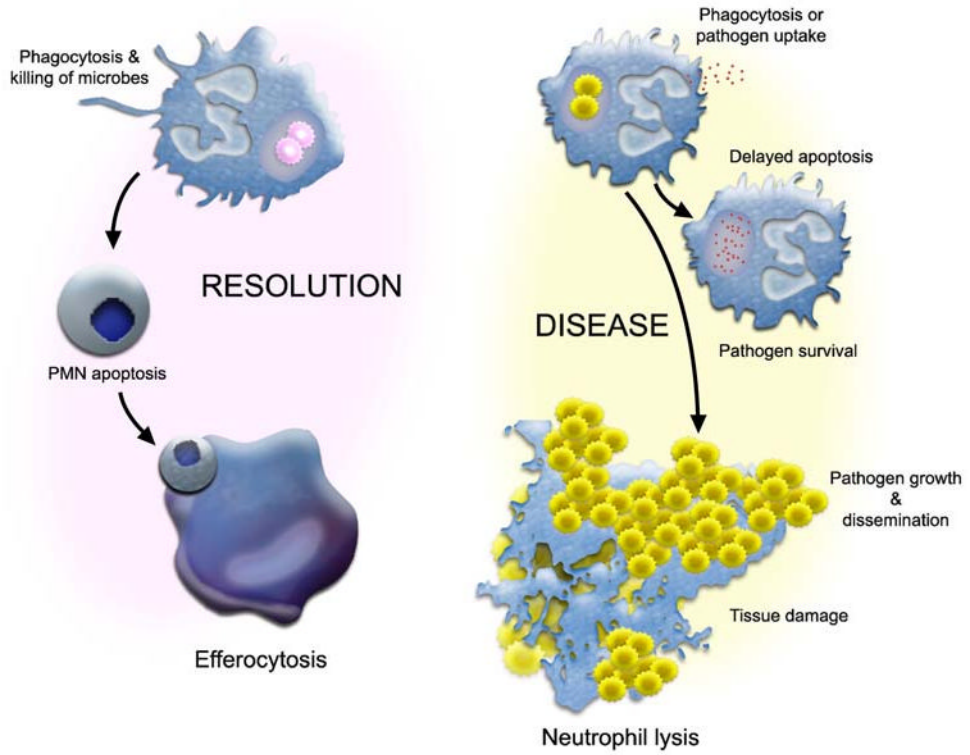

Fig. 1.

Two possible outcomes of the interaction of PMNs with bacteria. On one hand, bacteria (*pink spheres*) are ingested, activate PMNs, and are killed, and this process ultimately triggers PMN apoptosis and the removal of these cells by macrophages (*Efferocytosis*). This phenomenon leads to the resolution of the inflammatory response (*RESOLUTION*). Alternatively, bacterial pathogens (*yellow spheres* or *red particles*) are ingested and either delay normal PMN apoptosis and turnover or cause PMN lysis. Typically, these processes facilitate survival of bacteria and thereby promote disease (*DISEASE*)