Infection, Genetics and Evolution 21 (2014) 515–522

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Small colony variants (SCVs) of *Staphylococcus aureus* – A bacterial survival strategy

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ARTICLE INFO

Article history:
Available online 27 May 2013

Keywords:
Staphylococcus aureus
Small colony variants
Persistence
Intracellular
Survival strategy
Stress response

ABSTRACT

Small colony variants (SCVs) of *Staphylococcus aureus* have been implicated in chronic recurrent infections and have therefore gained renewed interest during the last decade. Moreover, SCVs have been shown to be part of the regular growth cycle, are highly dynamic or stable and can be selected during various harsh conditions. As such, the emergence of SCVs has been described not only in human, but also in veterinary medicine as well as in food microbiology. SCVs are characterized by impaired growth, down-regulation of genes for metabolism and virulence, while *sigB* and genes important for persistence and biofilm formation are up-regulated. Furthermore, SCVs are resistant to various antibiotics such as aminoglycosides, trimethoprim-sulfamethoxazole, fluorquinolones, fusidic acid or even to antiseptics such as triclosan. An underlying mechanism has been determined for hemin-, menadione- and thymidine-dependent SCVs as well as for SCVs which are impaired in their stress response. SCVs are optimized for persistence in the host. They are able to reverse and thereby constitute a highly dynamic subpopulation of *S. aureus*. Such phenotype switching constitutes an integral part of the infection process enabling the bacteria to hide inside the host cell without eliciting a strong host response.

1. Introduction

A first review of *Staphylococcus aureus* small colony variants (SCVs) dates back to 1935, when SCVs had first been thought to be part of the regular growth cycle (Swingle, 1935), and several reviews followed (Proctor et al., 2006; Sendi and Proctor, 2009; Melter and Radojevic, 2010; Atalla et al., 2011). More than 80 years later, the findings by Swingle et al. have been corroborated by Edwards. The author showed, that phenotypic switching between normal and SCV phenotypes is part of the exponential-phase growth without any selective pressure (Edwards, 2012).

In the last two decades, SCVs regained attention due to the observation, that these phenotypes are associated with persistent *S. aureus* infections, which are difficult to treat (Proctor et al., 2006). One important hallmark of SCVs is their small colony size on conventional agar plates including the decreased pigmentation of colonies and reduced hemolysis on blood-containing agar plates, which distinguishes SCVs from normal *S. aureus* isolates (Proctor et al., 1994). Such features complicate the correct isolation and identification for routine laboratories, which are mostly not trained to recognize these unusual phenotypes (Kipp et al., 2005). Thus, it can be assumed, that SCVs frequently remain unidentified during routine diagnostic. Also, conventional differentiation tests such as coagulase, catalase and biochemical tests are hampered by the decreased metabolism of these variants and often produce false negative results (Kahl et al., 1998). Moreover, commercial identification systems frequently fail to correctly identify these phenotypic variants. This is especially critical in the case of SCVs with methicillin-resistance (Kipp et al., 2004; Cleeve et al., 2006).

During the last years, the incidence of SCVs has been reported not only in various human clinical infections, but also in infections in veterinary medicine (Atalla et al., 2008) and in food microbiology (Karatzas et al., 2007; Onyango et al., 2012).

However, the term SCVs is merely descriptive with many conditions causing the induction and selection of SCVs. The genetic mechanism has been elucidated for only few SCVs. For many SCVs, the mechanism of emergence is still a matter of speculation. Ongoing research suggests that some SCVs might be induced by regulation of so far unrecognized important regulators and genes, because upon subculture, some SCVs tend to easily revert to the normal phenotype (Tuchscherr et al., 2011).

In this review, a brief summary of the current state of research on SCVs is provided. The review is separated into sections to accommodate the different status of knowledge about the mechanisms and the extremely diverse conditions leading to the SCV phenotype, the host immune response elicited by SCVs and recent research about new antibiotic treatment strategies.

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http://dx.doi.org/10.1016/j.meegid.2013.05.016

2. SCVs in different species

The occurrence of SCVs seems to be a natural survival mechanism for many, if not all bacteria, since SCVs have been described for several species including *Pseudomonas aeruginosa* (Haussler et al., 1999a,b), *Burkholderia cepacia* (Haussler et al., 2003), *Burkholderia pseudomallei* (Haussler et al., 1999a,b; Ramli et al., 2012), *Salmonella* (McCarthy et al., 1977), *Enterococcus faecalis* (Wellinghausen et al., 2009) and *Escherichia coli* (Roggenkamp et al., 1998). Most of these reports have in common that SCVs were isolated because of selective conditions such as antibiotic therapy, cold stress, exposure to disinfectants or the intracellular location in eukaryotic cells. Therefore, it is important to further study the underlying mechanisms causing these special phenotypes. If a general mechanisms can be identified for SCVs in different bacterial species, it may be possible to find phenotypic-directed treatment regimens to combat SCVs and hence, most likely also persistent infections.

3. *S. aureus* SCVs with defined auxotrophism

Some SCVs can be distinguished by supplementation with a particular substrate in a chemically defined medium. Supplementation either causes reversion of the growth to the normal phenotype or enhances the growth of the SCV surrounding a disc, which was impregnated with this particular substrate. Furthermore, the growth of some SCVs is supported under CO_2 (Gomez-Gonzalez et al., 2010). Depending on the specific substrate a mechanism for the emergence of such SCVs has been proposed. By this method menadione-, hemin-, thymidine-, and CO_2-dependent SCVs have been determined. The knowledge of the auxotrophism provides insights into the underlying mechanism causing the emergence of SCVs, which can be determined by sequencing of respective genes or operons (see below). However, there are still many SCVs, for which an appropriate auxotrophism has not been detected. New technologies such as whole genome sequencing (Takeuchi et al., 2005) and RNA-sequencing (Beaume et al., 2011; Lasa et al., 2011) are promising tools to elucidate underlying mechanisms for so far undefined SCVs if they are compared to isogenic wild-type strains.

3.1. Hemin- and menadione-dependent SCVs (electron-transport deficient SCVs)

The most advanced mechanisms leading to the SCV phenotype so far is the one described for hemin- and menadione-dependent SCVs, which arise after treatment with aminoglycosides (AGs) (Kohler et al., 2003; Balwit et al., 1994; Kaplan and Dye, 1976; Pelletier et al., 1979; Miller et al., 1978). AGs are positively charged antibiotics, which are transported into the bacterial cell in response to a high electrochemical gradient across the cytoplasma membrane (Baumert et al., 2002). Hemin- and menadione-dependent SCVs are resistant to AGs because of a low membrane potential, which prevents the uptake of AGs and other cationic substrates e.g. defensins or antimicrobial peptides such as lactoferrin B (Baumert et al., 2002; Samuelsen et al., 2005).

Interestingly, it has been shown *in vitro* that co-infection of *S. aureus* and *P. aeruginosa* also induced hemin-dependent SCVs by virtue of two different molecules that are produced by *P. aeruginosa*. The signal molecule 4-hydroxy-2-heptylquinoline-N-oxide (HQNQ) and pyocyanin are both secreted by *P. aeruginosa* and act upon respiration of *S. aureus* thereby inducing hemin-dependent SCVs (Hoffman et al., 2006; Biswas et al., 2009). Such hemin-dependent SCVs are resistant to AGs. These SCVs are protected against the effect of tobramycin, which is often used in CF patients to treat airway infections caused by *P. aeruginosa* (Doring et al., 2012), which might explain the increased prevalence of SCVs in older patients in worse clinical conditions (Besier et al., 2007a,b).

Another group of SCVs with resistance to fusidic acid (FA), which is an antibiotic used to treat *S. aureus* infections (Atkins and Gottlieb, 1999), has recently been described as also being hemin- or menadione-dependent (Norstrom et al., 2007).

3.2. Thymidine-dependent SCVs

Thymidine-dependent SCVs (TD-SCVs) are mostly related to chronic airway infection in CF patients (Gilligan et al., 1987; Kahl et al., 1998; Besier et al., 2007a,b; Yagci et al., 2013; Vergison et al., 2007), but can also be isolated from other infections, if patients have been treated for long periods with trimethoprim-sulfamethoxazole (Besier et al., 2008b). This antibiotic combination interferes with the synthesis of tetrahydrofolic acid, which functions as a co-factor for thymidylate synthase, an essential protein required for the conversion of thymidine from uracil (Stryer, 1995). TD-SCVs can only survive in the presence of external thymidine, which is provided in infected tissues because of cell degradation and pus (Besier et al., 2008b). In addition to the typical SCV growth described for all other SCVs, TD-SCVs can also exhibit what is referred to as a "fried-egg" colony appearance on Columbia blood agar plates (Kahl et al., 2003). In light and electron microscopy TD-SCVs display very heterogeneous cells with some large cells, and several or even not intact division planes indicating impaired cell division (Kahl et al., 2003).

3.3. SCVs with CO_2 auxotrophism

In a recent 3-year prospective study conducted in a University hospital in Spain, SCVs were collected from patients with different infections (Table 1) including respiratory and wound infections. The isolated SCVs were identified to be CO_2–dependent, which reverted after 3–6 sub-cultivations (Gomez-Gonzalez et al., 2010).

4. More conditions leading to the induction and selection of the SCV phenotype

4.1. SCVs induced inside eukaryotic cells

Infection of bovine endothelial cells with wild-type *S. aureus* displayed a high percentage of SCVs recovered from the intracellular milieu compared to wild-type bacteria that were not exposed to endothelial cells (Vesga et al., 1996). Recently, this observation was supported by *in vitro* and *in vivo* studies in long-term infection models using primary human umbilical endothelial cells and osteoblasts, which were infected for one week, respiratory epithelial cells and a haematogenous murine model, in which the infection lasted for 4 weeks (Tuchscherr et al., 2011). In addition, the authors determined the appearance of SCVs in clinical specimens from different persistent infections. All isolates from the infection models and from clinical infections revealed a high phenotypic diversity with an increase of SCVs over time indicating the induction and selection of SCVs during the intracellular location and persistent infection. However, after subculture most intracellular SCVs reverted to the wild-type phenotype thereby constituting a highly dynamic *S. aureus* population (Tuchscherr et al., 2011). Future research may focus on the impact of the various specific intracellular locations or components, which might act upon the induction of the SCV phenotype. In doing so, it may be possible to optimize the antibacterial therapy by interfering with the specific selective trigger.