nature reviews microbiology

https://doi.org/10.1038/s41579-023-00852-y

Review article

Check for updates

Staphylococcus aureus host interactions and adaptation

Benjamin P. Howden[1,2,3,4] ✉, Stefano G. Giulieri[2,5], Tania Wong Fok Lung[6], Sarah L. Baines[2], Liam K. Sharkey[2], Jean Y. H. Lee[2,7], Abderrahman Hachani[2], Ian R. Monk[2] & Timothy P. Stinear[1,2]

Abstract

Invasive *Staphylococcus aureus* infections are common, causing high mortality, compounded by the propensity of the bacterium to develop drug resistance. *S. aureus* is an excellent case study of the potential for a bacterium to be commensal, colonizing, latent or disease-causing; these states defined by the interplay between *S. aureus* and host. This interplay is multidimensional and evolving, exemplified by the spread of *S. aureus* between humans and other animal reservoirs and the lack of success in vaccine development. In this Review, we examine recent advances in understanding the *S. aureus*–host interactions that lead to infections. We revisit the primary role of neutrophils in controlling infection, summarizing the discovery of new immune evasion molecules and the discovery of new functions ascribed to well-known virulence factors. We explore the intriguing intersection of bacterial and host metabolism, where crosstalk in both directions can influence immune responses and infection outcomes. This Review also assesses the surprising genomic plasticity of *S. aureus*, its dualism as a multi-mammalian species commensal and opportunistic pathogen and our developing understanding of the roles of other bacteria in shaping *S. aureus* colonization.

Sections

[1]Centre for Pathogen Genomics, The University of Melbourne, Melbourne, Victoria, Australia. [2]Department of Microbiology and Immunology, The University of Melbourne, The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia. [3]Department of Infectious Diseases, Austin Health, Heidelberg, Victoria, Australia. [4]Microbiology Department, Royal Melbourne Hospital, Melbourne, Victoria, Australia. [5]Victorian Infectious Diseases Service, Royal Melbourne Hospital, The Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia. [6]Department of Paediatrics, Columbia University, New York, NY, USA. [7]Department of Infectious Diseases, Monash Health, Clayton, Victoria, Australia. ✉e-mail: bhowden@unimelb.edu.au

Introduction

Staphylococcus aureus is a frequent colonizer of the human population and one of the foremost opportunistic bacterial pathogens of humans, causing major morbidity and mortality globally[1]. Any clinician, especially those working in infectious diseases, will attest to the frequency, complexity and the potential catastrophic nature of invasive infections caused by this pathogen. Although on a global scale human colonization and infection with *S. aureus* are forefront of mind, the organism has a long evolutionary history as a multihost opportunistic pathogen. *S. aureus* colonizes approximately 20–30% of humans persistently in the nose[2] and frequently in other sites such as the skin, throat, axillae, groin and intestine[3]. The interplay between the potential pathogen and the host microbiota is being explored with increasing depth and complexity, highlighting roles for other commensals in shaping *S. aureus* colonization[4]. Colonization is harmless but it is a risk factor for developing subsequent infections (often caused by the colonizing strain[5]), which can range from mild skin and soft tissue infections to serious invasive infections, including osteomyelitis and septic arthritis, bacteraemia or septicaemia, pneumonia and endocarditis[1]. *S. aureus* infections can be acute, recurrent or chronic and persistent. To be best placed to develop effective therapies and clinical responses to these infections, we need to understand the complex pathophysiological relationships between *S. aureus* and its hosts[6].

The breadth of immune evasion mechanisms in *S. aureus* reflects the longstanding nature of the interaction of bacterium with humans, characterized by persistent colonization and intermittent invasive infections. *S. aureus* produces a large array of virulence and immune evasion factors that hinder the human immune response[7,8]. Neutrophils represent the major defence against staphylococcal infections. Staphylococcal factors that perturb adaptive B cell and T cell responses and diminish protective immunity are well described[8]. Factors subverting innate immune response also dominate, such as inhibition of neutrophil chemotaxis and killing, inhibition of complement activation and phagocytosis, killing of host cells and staphylococcal agglutination[6,8]. Reflecting the presence of factors inhibiting adaptive immune responses, infection with *S. aureus* does not elicit a protective immune response, meaning that recurrent infections are common throughout life[9,10]. Decades of vaccine studies with promising preclinical results have failed to translate into effective vaccines for use in human, including the failure of two major phase III clinical trials to demonstrate a benefit[11]. The adaptation of some *S. aureus* clones to the human host means that preclinical animal models are limited in utility[11].

In the colonization state, *S. aureus* is part of a polymicrobial community that primarily includes other staphylococci, corynebacteria and propionibacteria[12,13]. The roles of non-pathogenic commensals and interspecies interactions that influence *S. aureus* colonization and disease are largely unexplored[14]. However, advances in genomics-enabled microbial community analyses and metabolomics provide an opportunity to delve into these interactions (Supplementary Box 1 and Supplementary Fig. 1). In vivo *S. aureus* biofilms are more typically a monomicrobial community, in which surface-exposed proteins, referred to as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)[15], initiate the attachment to surfaces, further consolidated by the subsequent release of polysaccharides, proteins and extracellular DNA, forming an extracellular matrix encasing the bacteria. Biofilm-associated infections, which have a propensity to chronicity through increased antimicrobial resistance and immune evasion, are frequently linked to implanted devices and contribute to the pathogenesis of *S. aureus* infections in native tissues[15,16]. The mechanisms of immune evasion afforded by *S. aureus* biofilms have not been clearly elucidated, but are thought to be in part caused by the masking of pathogen molecular signatures[17].

Antimicrobial resistance acquired through acquisition of mobile genetic elements or chromosomal mutations has further complicated staphylococcal infections and therapeutics[18]. Although penicillin initially revolutionized the treatment of serious *S. aureus* infections, resistance through the acquisition of the β-lactamase encoding gene *blaZ* was soon widespread[19]. The acquisition of resistance to anti-staphylococcal penicillins through the *mecA* gene has resulted in the global dissemination of diverse lineages of methicillin-resistant *S. aureus* (MRSA), now considered a global public health threat[19]. Glycopeptides became the mainstay of therapy for invasive MRSA infections, but reduced susceptibility through adaptive mutations further hampered therapeutic efforts[20], which results in the move to using 'last-line' antimicrobials such as daptomycin, and combination therapies[19,20]. Pathogen evolution in cases of antimicrobial resistance and persistent human infections occurs in the milieu of the host environment, which imparts an additional selective pressure potentially selecting for not just drug resistance but also immune escape[21] (Supplementary Box 2 and Supplementary Fig. 2). The advent of accessible, high-throughput pathogen genomics technologies has revolutionized the study of staphylococcal resistance and adaptation, and the investigation of complex phenotypes linked to antimicrobial resistance, such as small colony variant (SCV) *S. aureus*[22]. The genomes of tens of thousands *S. aureus* isolates have been sequenced and are publicly available, and we are at an exciting stage in the utilization of this incredible resource for further understanding the pathogen[23]. Early use of the technology enabled the tracking of mutations that evolve during persistent clinical infections, which revealed an unexpected capacity for the pathogen to adapt to complex antimicrobial exposures and the host environment[24,25], and uncovered *S. aureus* genetic heterogeneity that can exist within host[26] (Box 1).

In addition to the growing list of newly discovered immune-modulating molecules, recent advances in our understanding of *S. aureus* pathogenesis and disease are highlighting the roles of biofilm formation, immunometabolic host interplay and immune evasion phenotypes (such as the SCV) to avoid host clearance. Added to this, *S. aureus* genomic studies are revealing a surprisingly high mutagenic potential that also materially contributes to immune evasion phenotypes. Finally, assessing bacterial adaptation processes occurring during switching between different vertebrate hosts is providing insights into host-specific disease mechanisms. In this Review, we examine and integrate recent key advances in understanding the mechanisms that *S. aureus* uses to cause infections. We explore our understanding of *S. aureus* immune evasion molecules and the role of biofilms, immunometabolism and *S. aureus* avoidance mechanisms of trained innate immunity. We also investigate *S. aureus* host specificity and adaptation and summarize current knowledge of the interplay between colonizing *S. aureus* and other bacteria with a summary of our emerging knowledge of *S. aureus* genomic plasticity, drug resistance and persistence. A detailed dissection of host responses, vaccines and other therapeutics is not covered in this Review.

Immune responses and immune evasion molecules

Staphylococci were first described in the 1870s, and staphylococcal leukotoxins were reported almost 100 years ago[27]. Despite intense study, the reasons for the apparent redundancy in these innate immune

Box 1

Staphylococcus aureus genome characteristics and summary of genetic changes enabling a switch from colonizer to pathogen and persistence

Staphylococcus aureus and its genome

S. aureus evolution within the host is driven by genetic variation generated within its small genome of 2.8–3.2 bp (encoding for about 2,500–3,000 proteins), which is carried by a single chromosome that can be associated with one or more plasmids[124]. The stable component (core genome) is complemented by a set of facultative genes (accessory genome) that can mediate antibiotic resistance, virulence or immune evasion mechanisms and are carried by mobile genetic elements[125]. Antibiotic-resistance genes are generally found on plasmids, transposons and the staphylococcal cassette chromosome (antibiotic and metal resistance genes), whereas phages and pathogenicity islands carry virulence and immune evasion determinants[126].

Molecular mechanisms of adaptation

Genetic diversity within the *S. aureus* genome arises from point mutations (SNP and InDels) and recombination (genome rearrangement through disruption and reconnection of chromosome fragments), which can lead to chromosome structural variants such as inversions, duplications, large insertions and deletions (Supplementary Table 2 and Supplementary Fig. 2). In addition, *S. aureus* can acquire foreign DNA via mobile genetic elements, although this phenomenon is only relevant during colonization[127]. The recombination or mutation ratio is thought to be low in *S. aureus*[128] and may be even lower when considering within-host evolution[127], which indicates that point mutations are the major drivers of diversity within the host. This is also highlighted by higher mutation rates in within-host studies when compared with population genomics studies and by the peculiar role of hypermutators in certain settings, such as chronic carriage associated with cystic fibrosis[129]. However, chromosome structural variants are recurrently observed within the host and seem to be particularly important during infection as opposed to colonization[130]. These changes include loss of large genome segments (prophages or pathogenicity islands[131]), chromosome duplications and mobilization of insertion sequences. The overall preponderance of deletions and gene disruption has suggested that infecting strains undergo reductive evolution as they adapt to the intracellular environment as observed for other pathogens[132,133].

Within-host evolutionary models

According to the standard model of *S. aureus* within-host evolution, colonization is initiated by transmission to a new host of a distinct clone followed by spread and niche expansion on colonization sites[113]. Co-colonization with genetically distant lineages is estimated to occur in about 5% of the cases[130,134], but can be as high as 45% in patients with cystic fibrosis[135]. Transition from colonization to infection (for example, bacteraemia) occurs rarely and can be followed by dissemination and establishment of secondary foci. In what can be considered a substantial change of ecological niche, with exposure to different selective pressures (for example, high-dose antibiotics, immune response or nutrient sequestration), the invasion of *S. aureus* into the bloodstream and organs is effectively an evolutionary dead-end. These events are shaped by several population bottlenecks (at transmission, at transition from colonization to infection and on organ seeding during bacteraemia)[112], which indicates that genetic drift underlies a substantial proportion of the genetic changes observed. However, several lines of evidence from within-host evolution studies support a role of adaptive evolution within the host during infection. First, mutations that affect protein function (non-synonymous and protein-truncating) are enriched over synonymous mutations[131,136]. These conclusions are supported by strong evidence of convergent evolution with recurrent acquisition of mutations in key *S. aureus* loci. The most important of these adaptive loci is *agr*, the master regulator of *S. aureus* virulence, which is frequently switched off within the host[130,137,138]. Besides *agr*, there are other key core genes targeted by adaptive evolution that have regulatory functions: these include the essential cell-wall regulatory system *walKR*[24], *rsp* (transcription factor repressor of surface proteins)[136,139] and the adaptor protein *yjbH*[130,140], that interacts with the transcriptional regulator encoded by *spx*[141]. Recently, within-host genomic studies have also indicated that metabolic genes such as *sucA-sucB* are hotspots of bacterial host adaptation[130,142,143]. These adaptive processes seem to be specific to colonization and infection. For example, *agr* and *rsp* variants dominate in strains that adapt from colonization to infection, whereas mutations in regulatory genes that influence antibiotic resistance and immune evasion are found in persistent strains and metabolic genes are mostly mutated in adapted colonizing lineages[130].

Phenotypic correlates of adaptation in *S. aureus* infections

Phenotypic adaptation is recognized as a key marker of persistent *S. aureus* carriage in patients with cystic fibrosis and persistent bloodstream and skeletal infections. Importantly, this phenomenon is sometimes observed in only a small proportion of the infecting strains (heterogenous phenotype) or is associated with frequent alternation between two phenotypic states (phase variation)[103]. Classic adaptive phenotypes include secondary antibiotic resistance (in particular subtle changes such as vancomycin-intermediate *S. aureus* and heterogeneous vancomycin-intermediate *S. aureus*)[25,144,145] and small colony variants[146,147]. Recently, reduced eukaryotic cell toxicity was recognized as an important adaptive phenotype related to invasive *S. aureus* infections with a case of loss of toxicity in late colonizing strains that leads to infection with a low-toxicity strain[148]. The adaptation was linked to mutations in *rsp*, mimicking *agr* inactivation[139]. Some immune evasion phenotypes can also be observed in within-host adapted strains, including reduced

(continued from previous page)
whole-blood killing and resistance to antimicrobial peptides[21,149]. Although these adaptations often lead to reduced mortality in animal models, secondary mutations in adapted low-toxicity strains can restore virulence[138], which highlights the complexity of toxicity trade-offs during infection. Finally, metabolic adaptation has been recently documented in persistent bacteraemia with convergent mutation in citrate metabolism genes associated with metabolic antibiotic resistance[143].

effector molecules secreted by *S. aureus* have remained uncertain, partly owing to a lack of detailed understanding of the molecular mechanisms of action[7]. Nonetheless, new immune evasion factors, including extracellular proteins and polysaccharides, are still being discovered and refined mechanistic insights continue to be gained (Fig. 1 and Supplementary Table 1). The large number of immune evasion factors found in *S. aureus* contrasts with many other bacterial pathogens that often produce a smaller number of specific factors[28] and probably reflects the long-standing and complex relationship between *S. aureus* and its mammalian hosts.

Neutrophils are critical in the defence against infection from *S. aureus*[8], with chronic granulomatous diseases caused by defects in neutrophil killing and recurrent *S. aureus* infections being a key example[29]. *S. aureus* targeting of neutrophils spans multiple arms of bactericidal activities, including chemotaxis, opsonophagocytosis and neutrophil-mediated killing and staphylococcal killing of host cells, including neutrophils[6,8].

Recently discovered immune evasion molecules

A large repertoire of well-characterized molecules contributes to the survival of *S. aureus* following phagocytosis, in which the bacterium is exposed to several toxic products such as reactive oxygen species, nitric oxide, host antimicrobial peptides and neutrophil serine proteases (NSPs)[30,31]. The extracellular adherence protein (Eap) and homologues (EapH1 and EapH2) secreted by *S. aureus* can non-covalently inhibit NSPs at low nanomolar concentrations[31] (Fig. 1). The targeted inhibition of NSPs prevents bacterial killing, regulation of neutrophil extracellular traps (NETs) and the degradation of phenol-soluble modulins (PSMs)[32]. PSMs target the neutrophil formyl-receptor 2, which leads to degranulation and neutrophil lysis[32]. In addition to PSMs, NSPs can target, degrade and functionally inactivate a range of staphylococcal immune evasion factors, including chemotaxis inhibitory protein of *S. aureus* (CHIPS) and staphylococcal complement inhibitor-A. However, the activity of these proteases seems different against homologues of immune evasion proteins with similar functions, possibly providing some explanation for the redundancy in the large number of immune evasion factors secreted by *S. aureus*[33]. All three Eap proteins were shown to protect against the activity of NSPs against staphylococcal immune evasion factors[32,33] (Fig. 1).

Within the phagosome, myeloperoxidase (MPO) uses H_2O_2 as a substrate to generate highly toxic reactive oxygen species that may act synergistically with intracellular proteases against *S. aureus*[34]. High-throughput screening recently identified an additional factor inhibiting neutrophil killing through specific binding and inhibition of MPO, called staphylococcal peroxidase inhibitor[35]. Expression of this molecule is upregulated following phagocytosis and contributes to evasion of MPO-dependent killing[35] (Fig. 1). Like other human immune evasion factors secreted by *S. aureus*, staphylococcal peroxidase inhibitor does not bind to, nor inhibit MPOs from other species such as mouse, rabbit or cow. The host specificity adds additional challenges investigating in vivo activity of these virulence factors[36].

Superantigens (sAgs) are bacterial toxins that result in T cell proliferation and immune dysregulation, contributing to the pathogenesis of invasive *S. aureus* infections and having a role in colonization. sAgs bind to a large repertoire of variable-β chains in the T cell receptor. Toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxins B and C are the most well described of these molecules[8] (Supplementary Table 1). However, the family of staphylococcal sAgs numbers over 26 (ref. [37]), which facilitates binding to a large repertoire of variable-β chains in the T cell receptor. Large-scale genomic analyses are further refining the distribution and identification of staphylococcal sAgs[37]. Recently, staphylococcal enterotoxin-like toxin X (SElX) has been identified as an additional sAg that is encoded in the genome of 95% of *S. aureus* isolates examined. SElX has been shown to induce T cell activation, which contributes to necrotizing pneumonia in a rabbit model[38] (Fig. 1). SElX has a mechanistically independent contribution to phagocytosis inhibition by binding neutrophils via neutrophil surface receptors, providing an example of a staphylococcal sAg with both innate and adaptive immune disruptive strategies[39]. Genomics recently identified the staphylococcal sAgs SElW in 97% of the isolates studied[37]. The gene-encoding SElW (*selw*) has been truncated in several staphylococcal clones but full length in others, including the human and livestock-adapted clone CC398. Functional analysis revealed that SElW is a potent T cell activator contributing to pathogenesis in a bacteraemia model and that this sAg is solely responsible for T cell mitogenic activity of CC398 (ref. [37]).

New insights into previously described molecules

Many cell-wall-anchored adhesins are expressed by *S. aureus*, including those making up the MSCRAMM family[40]. The serine–aspartate repeat protein D (SdrD) is a major MSCRAMM protein that contributes to colonization and infection, through attachment to the nasal epithelium[41], and abscess formation[42]. Recently, this protein has been shown to inhibit innate immune-mediated killing of *S. aureus*, while promoting bacterial survival in human blood; however, the molecular mechanisms supporting these activities have not been elucidated[43]. Another serine–aspartate repeat protein, SdrE, has also recently been found to bind complement factor H, which leads to complement evasion[44] (Supplementary Table 1). The fibronectin-binding protein FnbpB has an essential role in binding to host ligands, platelet activation and invasion into non-phagocytic cells[45]. In addition to the binding of plasminogen that can then be converted to plasmin by host activators or endogenously expressed staphylokinase, a study recently identified a novel role for FnbpB in the binding of histones (which are a major antibacterial component of NETs), which leads to their degradation[46].

Host deployment of NETs is a remarkably efficient defence mechanism against bacterial intrusions, albeit leading to neutrophil death (NETosis). In response, *S. aureus* has evolved a two-pronged counterattack. By secreting the staphylococcal nuclease Nuc, *S. aureus* degrades the NETs DNA backbone and uses adenosine synthase A (AdsA), which is a cell-wall-anchored enzyme that converts extracellular ATP and ADP into

the nucleoside Ado, an anti-inflammatory molecule[47,48]. AdsA also converts dAMP into deoxyadenosine (dAdo), a potent inducer of caspase-3-dependent apoptosis in macrophages, thus AdsA has multiple roles blunting macrophage efferocytosis activity at the infection site (Fig. 1).

Another important class of immune evasion molecules in *S. aureus* consists of the bicomponent pore-forming toxins (leukocidins), which to date have been primarily linked to neutrophilia (also called neutrophil leukocytosis), although these toxins target a range of other

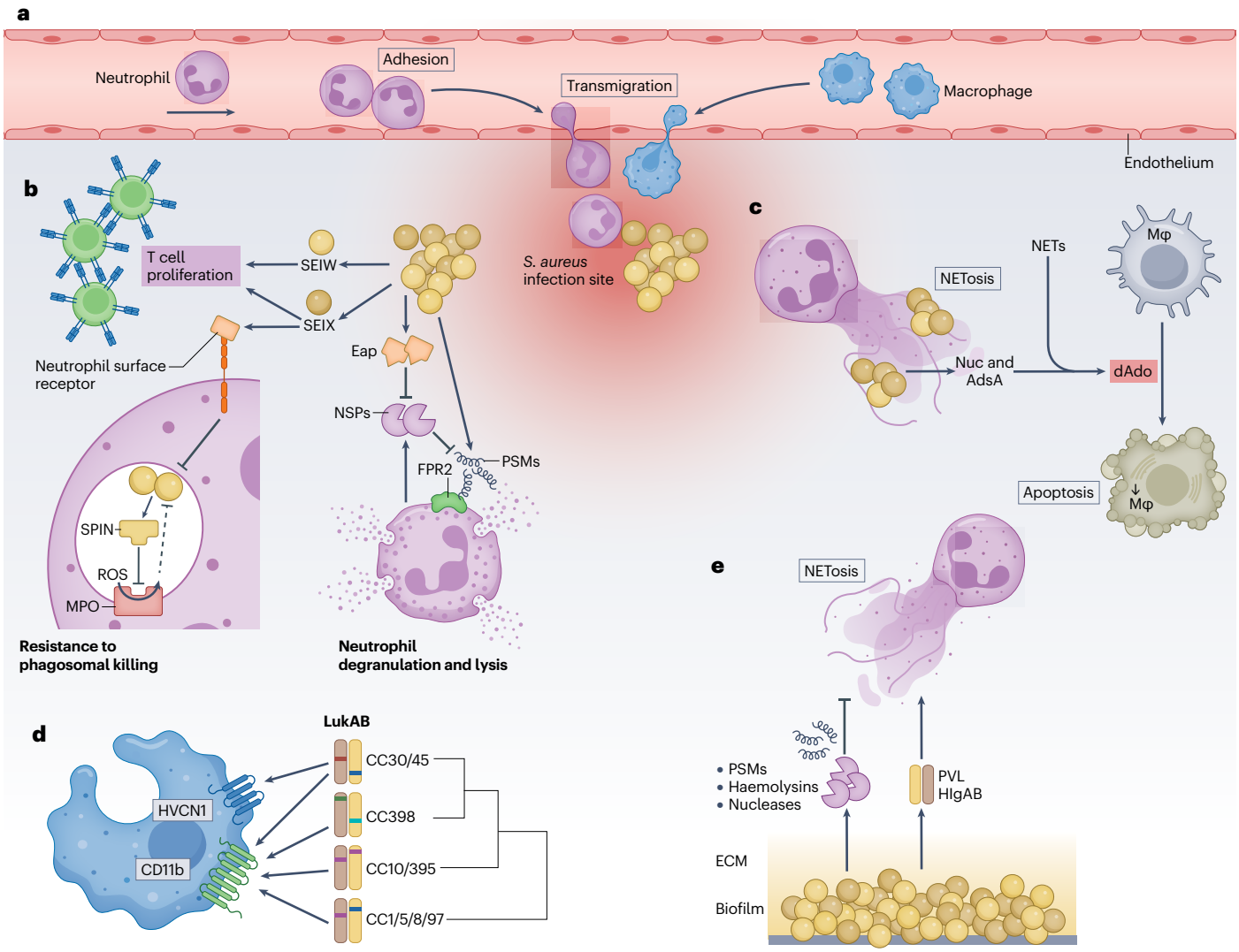


Fig. 1 | New insights into *Staphylococcus aureus* immune evasion. **a**, Recruitment of neutrophils and phagocytic cells to the site of infection. Vascular neutrophils and phagocytic cells, such as macrophages, are actively recruited by chemotaxis to *Staphylococcus aureus* infection site following chemokine gradients and sensing pathogen-associated molecular patterns and bacterial-derived ligands of the formyl-peptide receptors released by *S. aureus*. **b**, Recently described *S. aureus* immune evasion molecules. *S. aureus* secretes phenol-soluble modulins (PSMs) that cause neutrophil lysis through interaction with formyl-receptor 2 (FPR2). Secreted neutrophil serine proteases (NSPs) degrade PSMs, but this process is inhibited by *S. aureus* extracellular adherence protein (Eap; or orthologues EapH1 and EapH2). *S. aureus* secretes a range of superantigens including staphylococcal superantigen (SEIW) and the bifunctional staphylococcal enterotoxin-like toxin X (SEIX) that lead to T cell proliferation and immune dysregulation. SEIX also binds to neutrophil surface receptors and inhibits phagocytosis. Within the phagosome, myeloperoxidase (MPO) contributes to phagosomal killing through the generation of reactive oxygen species (ROS). However, *S. aureus* secretes staphylococcal peroxidase inhibitor (SPIN) that inhibits this process. **c**, Disruption of macrophage efferocytosis. *S. aureus* can indirectly interfere with macrophage efferocytosis of neutrophils undergoing formation of neutrophil extracellular traps (NETosis), whereby *S. aureus* neutralizes the bactericidal activity of neutrophil extracellular traps (NETs) using staphylococcal nuclease (Nuc) and adenosine synthase A (AdsA). The activities of these enzymes generate deoxyadenosine (dAdo), which triggers macrophage apoptosis. **d**, Genetic heterogeneity in virulence factors can alter their function. Clonal complex-specific sequence heterogeneity in the leukocidin AB (LukAB) leukotoxin alters phagocyte receptor tropism and cytotoxicity profile. This is exemplified by the strains belonging to CC30(CC45), which gained the ability to bind human hydrogen voltage-gated channel 1 (HVCN1). **e**, *S. aureus* biofilms secrete immune evasion factors to promote and inhibit NETosis. Secreted factors from *S. aureus* biofilms include leukocidins Panton–Valentine leukocidin (PVL) and *S. aureus* γ-haemolysin (HlgAB) that promote NETosis as well as nucleases that degrade NET DNA. CD11b, cluster of differentiation molecule 11B; ECM, extracellular matrix; Mφ, macrophage.

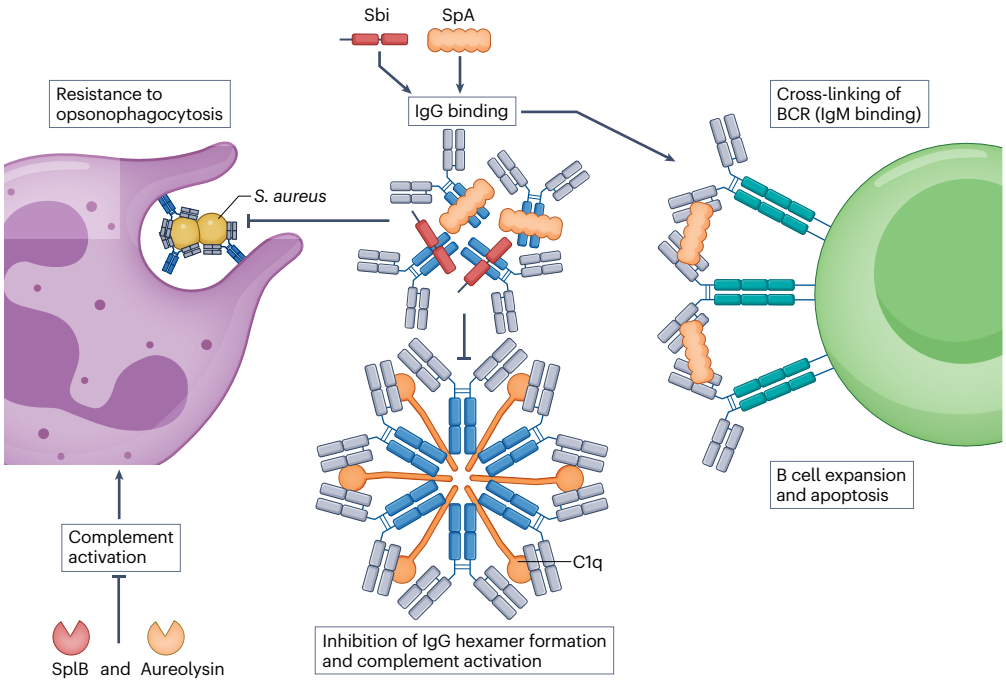


Fig. 2 | Evasion of immunoglobulin and complement-mediated immunity. Binding of IgG Fc domain by secreted protein A (SpA) and staphylococcal immunoglobulin-binding protein (Sbi) protects *Staphylococcus aureus* from phagocytosis and inhibits complement activation. Protein A also functions as a B cell superantigen, binding to the Fab domain of IgM and cross-linking V_H3-type B cell receptors (BCRs). In conjunction with aureolysin, the secreted protease serine protease-like protein B (SplB) degrades complement components, thus hampering complement activation and opsonophagocytosis. C1q, complement component 1q.

immune cells[7]. Leukocidins share a highly conserved structure, with chromatography elution profiles differentiating fast (F) and slow (S) components that form octameric membrane-spanning pores (Supplementary Table 1). Different leukocidins bind to specific cell receptors, which cause cell lysis after pore formation[7] (Fig. 1). There is substantial interest in further defining the action of these major toxins in disabling the axis between innate and adaptive immune responses to *S. aureus* infection. By deploying leukocidin AB (LukAB), *S. aureus* kills dendritic cells, thereby preventing antigen presentation necessary for the establishment of adaptive immunity[49]. However, the cell specificity of LukAB is still under investigation, which will be important for the future development of immune-mediated prevention strategies[49].

To date, the leukocidins have been studied as a 'class', and there is increased interest in understanding if their amino acid sequence heterogeneity may contribute to differential action. Greater sequence variation in LukAB compared with other leukocidins has been described[50], and recent genomic interrogation of *lukAB* highlighted sequence variations for this toxin, specific to *S. aureus* lineages[51]. Although binding of the LukAB toxin to phagocytes is mediated by cluster of differentiation molecule 11B (CD11b), it has also been shown that LukAB derived from two major *S. aureus* clonal complexes (CC30 and CC45) are cytotoxic to CD11b-depleted human monocytes[51]. The human hydrogen voltage-gated channel 1 was identified as the alternate target for LukAB produced by these clonal complexes[51].

The resistance of mouse phagocytes to most leukocidins that target human phagocytes has hampered research into the activity of these toxins[7]. The S-component of the toxins is the major molecule that demonstrates human cell-receptor specificity, and for protein subunits S and F of Panton–Valentine leukocidin (LukSF-PV) this is the human complement C5a receptor 1 (hC5aR1)[52]. Use of high-resolution microscopy suggests that hC5aR1 may dissociate from the toxin ligand after pore formation providing an additional receptor for other ligand binding and potential amplification of the inflammatory response[53]. This observation has implications for the modes of action of other bi-component toxins if the process is generalizable. Although binding of the S-component of LukSF-PV to hC51R1 explains human phagocyte specificity, a CRISPR–Cas9 screen identified CD45 as a specific target of the F-component[54]. Thus, although mouse models have proven very useful in dissecting host–staphylococcal interactions, their limitation, particularly pertaining to human specificity of virulence factors, has led to the development of humanized mouse models with increased susceptibility to *S. aureus* infection[55–59].

Although numerous studies have investigated the mechanisms of action of staphylococcal toxins and immune evasion factors, linking specific factors to disease manifestations has not always been straightforward. This area of research is complicated by the large repertoire of bacterial factors and apparent redundancy as described earlier. However, as access to bacterial genome sequence data has increased, statistical genomics approaches such as genome-wide association studies and machine learning have begun to link genome traits with *S. aureus* disease manifestations[60]. For example, the role of LukFS-PV in staphylococcal disease was heavily debated following the global emergence of community-MRSA clones that frequently carried genes encoding this toxin. Although the contribution of this toxin to purulent skin diseases is well established, the role of the toxin in other manifestations such as pyomyositis was disputed[61]. The use of genome-wide association studies linking bacterial genome data to clinical data on diseases manifestations has now shown that LukFS-PV is a critical toxin in the manifestation of pyomyositis in children[61].

S. aureus also produces a subset of proteins called sAg-like proteins (SSLs) that have a range of immunomodulatory functions, including inhibition of chemotaxis and phagocytosis[8], although the function and full range of biological activities for all SSLs are not known. Through a broad range analysis of staphylococcal molecules that inhibit matrix metalloproteinases, endopeptidases involved in inflammatory cell recruitment and migration, SSL1 and SSL5 were identified as broad

Box 2

Overview of recent data on the immunogenetics of *Staphylococcus aureus* infections

The variability in clinical presentation and outcome among individuals with invasive *Staphylococcus aureus* infections is poorly understood. Although many *S. aureus* infections seem to occur in humans without major immune deficiencies, recent immunogenetics studies may be challenging this assumption. In these studies, human genetics are linked to infectious diseases manifestations to uncover the genetic determinants of the disease. A link has been identified between increased variation and reduced expression of *GLS2* in individuals with complicated *S. aureus* bacteraemia[150]. A recent genome-wide association study investigating human genetics of severe staphylococcal diseases found enrichment for rare heterozygous *OTULIN* variants in patients with severe disease. The gene encodes an enzyme, with the heterozygous variant leading to haploinsufficiency that results in enhanced cellular susceptibility to α-haemolysin toxin[151]. Another recent study interrogated DNA methylomes between individuals with and without persistent *S. aureus* bacteraemia and identified divergent DNA methylation signatures in patients with persistent bacteraemia, which suggests that epigenetic profiling may be useful for disease risk stratification[152].

range inhibitors of matrix metalloproteinases, hindering the motility and chemotaxis of neutrophils[62]. This represents the first identified role of SSL1 and identifies an additional role for SSL5 (ref. [63]). Recently, a second function was identified for SSL1 exhibiting in vitro protease activity and in a rabbit ocular infection model[64]. The study of the contributions of SSLs to immune evasion and pathogenesis has been hampered because SSLs are not expressed in mouse infection models. Overexpression vectors have been used to study SSLs in these models and have confirmed a role for SSL3 in staphylococcal pathogenesis via toll-like receptor 2 inhibition[65]. Contrary to the understanding that SSLs function as immune evasion factors, it seems that one SSL at least (SSL13) induces neutrophil responses following infection via the formyl-peptide receptor 2 to stimulate attraction and activation of neutrophils[66]. This result further highlights the complex interactions of immune modulating molecules during *S. aureus* infection.

Antibodies are key to the human immune response against bacterial infections, through direct bacterial binding, which results in complement activation through the formation of IgG clusters[67]. All clinical *S. aureus* strains produce protein A (SpA), which is a sortase-anchored surface protein initially deposited in the cell envelope and released through activity of LytM, a cell wall hydrolase[68], and inefficient sorting[69]. SpA binds the Fc region of IgG and the Fab domain of antibodies (Fig. 2). Binding to the Fc region of IgG hampers phagocytosis[70], whereas binding to the Fab domains and cross-linking of B cell IgM receptors lead to B cell expansion and apoptosis, tempering adaptive immune responses against *S. aureus*[8] (Fig. 2). More recently, the inhibition mechanism of complement activation by SpA has been described under highly purified conditions. Binding of soluble SpA to IgG was found to inhibit Fc–Fc contacts, thereby inhibiting IgG hexamerization and thus hindering complement activation[71]. Interestingly, *spa* expression and SpA production were more increased in a hospital-adapted clone of *S. aureus* (sequence type [ST]239 MRSA) than in a clone that is emerging in the community (ST398 MRSA), which suggests that higher expression in ST239 MRSA may contribute to the colonization and immune evasion phenotypes observed clinically[72]. Like Spa, the staphylococcal immunoglobulin-binding protein (Sbi) binds the Fc region of IgGs with high affinity via two surface exposed globular domains[73]. The release of Sbi exposes two additional domains that directly interfere with the activation of the alternative complement pathway[74]. Components of the complement cascade are also the targets of *S. aureus* serine protease-like B protein and the metalloproteinase aureolysin, which are active in human serum and shown to block neutrophils opsonophagocytosis[75] (Fig. 2).

Access to large-scale human genome sequencing capacity is also starting to reveal host genetic factors linked to staphylococcal diseases (Box 2). How our deepening understanding *S. aureus* immune evasion is starting to inform new therapeutic exploration is discussed in Box 3.

Role of biofilms and immunometabolism

In addition to the host evasion factors described in the previous section, biofilm formation is another mechanism that contributes to the pathogenesis of *S. aureus*[76]. Biofilms on biomedical devices, such as catheters and medical implants, or on host tissues such as wounds or heart valves cause notoriously difficult-to-treat infections[77]. By embedding bacteria in an extracellular matrix consisting of proteins, polysaccharides and extracellular DNA, biofilms confer protection against antimicrobial therapeutics and host immune clearance[77]. Nonetheless, secretion of factors including haemolysins, nucleases and PSMs are also important mediators of host evasion during the biofilm lifestyle and could shift immune responses towards an anti-inflammatory state[78,79].

The contribution of the leukocidins Panton–Valentine leukocidin (PVL) and *S. aureus* γ-haemolysin (HlgAB) to bacterial survival in biofilms was demonstrated using a porcine wound model[17]. High-level secretion of PVL and HlgAB in biofilms elicited the formation of NETs by neutrophils (Fig. 1). Although NETs trapped and killed planktonic bacteria, they had no activity against *S. aureus* in biofilm[17]. *S. aureus* nuclease (Nuc) degraded the DNA content of NETs, which is likely to have promoted the dispersal of staphylococcal cells from the biofilm, increasing the risk of metastatic infections[76]. Additionally, LukAB has been shown to contribute to evasion of phagocyte-mediated killing of *S. aureus* in biofilm[76].

S. aureus biofilms skew the host immune response towards an anti-inflammatory state, thereby promoting bacterial persistence instead of clearance (Fig. 3). This is evidenced by the recruitment of myeloid-derived suppressor cells (MDSCs)[80–83] and the polarization of anti-inflammatory M2-like macrophages[84]. MDSCs are typically prominent in tumour microenvironments and notorious for their immunosuppressive effect, inhibiting T cell activation and promoting tumour progression (reviewed elsewhere[85]). Analysis of purified MDSCs recovered from *S. aureus* biofilm-associated orthopaedic infection revealed increased expression of anti-inflammatory mediators such as arginase 1 and IL-10 (ref. [82]). Furthermore, depletion of MDSCs promoted bacterial clearance[82].

Typically, macrophages become activated on recognition of bacteria, with the ensuing inflammatory response driven by major cellular metabolic changes, a process termed immunometabolism[86]. These changes include increased host cell glycolysis, impaired tricarboxylic acid (TCA) cycle and mitochondrial oxidative phosphorylation (OXPHOS), all

Box 3

How greater understanding of *Staphylococcus aureus* is driving new therapeutic research

Antivirulence strategies

Because of the important role that *Staphylococcus aureus* virulence factors have in disease, they present attractive targets for novel therapies, especially in an era of widespread antimicrobial resistance. On the basis of the understanding of these mechanisms, a number of novel therapies have been developed and investigated. Such approaches could be used alone or in combination with antibiotic therapy[153]. As examples, progress has been made in the development of monoclonal antibodies[154,155], or biological agents such as centyrins (small proteins derived from fibronectin type III-binding domains)[156] that bind pore-forming toxins to neutralize bicomponent leukocidins. Small-molecule inhibitors have been developed with activity against the *S. aureus agr* quorum sensing system[157] and to target immune evasion factors such as staphyloxanthin[158]. Understanding the role of protein A in capturing immunoglobulins and preventing their effective action against *S. aureus* has led to the development of therapeutic antibodies against *S. aureus* that are engineered to avoid sequestration by protein A[159]. Targeting virulence factors as potential vaccine targets has failed in human clinical trials[160], with recent data showing that previous *S. aureus* exposure modulates the humoral response and leads to the vaccine being non-protective[161]. Further understanding this modulation may identify more successful *S. aureus* vaccine strategies.

Probiotics and microbiota-inspired therapies

Insights into microbiota–*S. aureus* interactions are inspiring new therapeutic endeavours, including the use of the human commensal microorganism *Staphylococcus hominis* as bacteriotherapy in atopic dermatitis[162] and as a potential therapy for *S. aureus*-associated wounds[163]. Probiotic *Bacillus* spp. may be used to eradicate *S. aureus* gut colonization, whereas naturally occurring quorum-sensing inhibitors could be examples of purified compounds that could be used for the treatment of infections[164]. These approaches are very early in their development.

Immunometabolic therapy

Increasingly, severe infections are considered as an immunometabolic dysfunction, with therapeutic considerations seeking to alleviate the hyperinflammation (cytokine storm) in critically ill patients. This strategy limits damage to the host and promotes host survival (disease tolerance). This has prompted the development of newer therapeutic agents that target immunometabolism, including the potential use of itaconate derivatives to dampen inflammation[165]. Similarly, the pivotal role of immunometabolism and accumulated metabolites in determining the outcome of infection following staphylococcal infection has been demonstrated in various tissue sites (reviewed elsewhere[166,167]). For example, staphylococcal biofilms skew macrophage metabolism towards mitochondrial oxidative phosphorylation (OXPHOS), which promotes an anti-inflammatory state and enables bacterial persistence[168]. Redirecting macrophage metabolism from OXPHOS to glycolysis via the delivery of nanoparticles containing the OXPHOS inhibitor oligomycin augmented inflammation and coincided with reduced biofilm burden in a mouse model of prosthetic joint infection[84]. This indicated that metabolic remodelling could prove an effective therapeutic strategy in the treatment of persistent staphylococcal infection. Although these approaches are still in their infancy, the emerging field of immunometabolism opens an exciting new area in therapeutic research.

Approaches to use pathogen genomics for patient management

Building on the recognition that adaptive evolution drives important clinical outcomes in *S. aureus* infections including both invasiveness of colonizing strains[137], and persistence and treatment failure in invasive strains[130,143], approaches to detect mutation in high-risk adaptive genes could support clinical decision making. This strategy has been successfully used in cancer medicine, whereby genomics approaches are used to detect driver mutations[169]. To be implemented in the management of *S. aureus* infections, large-scale studies are needed to establish the association between adaptive mutations and clinical outcomes[170,171]. However, bacterial genome-wide association studies of clinical outcomes have so far failed to show robust associations, possibly because of small sample sizes[172,173]. Unlike cancer, it is possible that adaptive mutations are relevant only in distinct infection types with higher risk of driving evolutionary changes owing to high bacterial burden and persistent infection nidus[130]. Although theoretically quite easily implementable into clinical practice, the lack of distinct *S. aureus* mutation signatures that would inform specific therapeutic decisions (apart from antimicrobial-resistant detection) means that this approach is still in the exploratory phase.

Insights from human genomics studies and multi-omics profiling

Human genomic studies that are more accurately defining the human genetic determinants of severe diseases severity and infection persistence could potentially identify targeted therapeutics. For example, in individuals with *OTULIN* deficiency, antibodies against α-toxin protected human cells and compensated for the genetic impact[151]. In other patients, identifying genetic signatures linked to persistent infection could inform different therapeutic approaches[152]. A recent study of early *S. aureus* bacteraemia multi-omics profiles identified predictive signatures that could also potentially be used clinically[174]. Substantial additional research is required before these types of approach would be available in a clinically relevant time frame.

resulting in the cellular accumulation of succinate and itaconate, which are two TCA metabolites[87,88]. Succinate is a pro-inflammatory metabolite that stabilizes the transcription factor, hypoxia-inducible factor 1α, which enhances the production of the pro-inflammatory cytokine IL-1β; whereas itaconate is an anti-inflammatory metabolite that restores homeostasis following succinate-mediated inflammation[87,88]. Itaconate exerts its immunomodulatory role by alkylating cysteine residues and inhibiting several targets including the NLRP3 inflammasome, glycolytic enzymes and succinate dehydrogenase (SDH) mitochondrial complex II[88–90]. Interestingly, rather than stimulating inflammatory macrophages, *S. aureus* biofilms induce the polarization of macrophages into an anti-inflammatory state, with a metabolic bias towards increased OXPHOS and decreased aerobic glycolysis[84] (Fig. 3). Importantly, the delivery of nanoparticles containing oligomycin, which is an OXPHOS inhibitor, in a mouse model of prosthetic joint infection resulted in the polarization of inflammatory monocytes and enhanced bacterial clearance. This demonstrates the potential of immunometabolic therapy in treating persistent staphylococcal infections (Box 3).

The increased expression of anti-inflammatory IL-10 by monocytes and MDSCs recruited to biofilms has been attributed to staphylococcal lactate, a metabolite that inhibits histone deacetylase 11 (HDAC11), thus enhancing IL-10 gene transcription[91]. This example highlights the crucial contribution of active bacterial metabolism and its crosstalk with host metabolic activities in determining the outcome of infection. Although mechanisms promoting immune evasion in biofilm-associated infections are being revealed[92], the factors driving *S. aureus* biofilm formation in vivo are poorly defined. A recent study exploring the role of host immunometabolism during *S. aureus* pulmonary infection identified the inhibitory effect of the host metabolite itaconate on bacterial glycolysis as a biofilm-promoting cue[93].

Itaconate is produced by the enzyme ACOD1 (also known as IRG1) in myeloid cells, particularly in response to lipopolysaccharide stimulation[94]. Of note, despite lacking lipopolysaccharide, *S. aureus* induces the production of host itaconate via the stimulation of mitochondrial oxidant stress[93]. Itaconate in turn inhibits the staphylococcal glycolytic enzyme aldolase, mirroring one of its interactions in mammalian cells[90]. Given the preferential reliance of *S. aureus* on glucose consumption and glycolysis for growth from carbon catabolite repression, the inhibition of glycolysis by itaconate rewires *S. aureus* metabolism, directing carbon flux towards biofilm formation and a sessile lifestyle. In an infection context, the consequence of metabolic adaptation for biofilm formation was revealed in a longitudinal study of *S. aureus* isolates colonizing the airways of a patient with cystic fibrosis over 15 years. These isolates displayed increasing production of biofilms in the presence of itaconate, consistent with the role of this metabolite in driving bacterial adaptation via biofilm formation[93]. Although the mechanism through which itaconate promotes persistent staphylococcal pulmonary infection and its clinical relevance requires further exploration, this study provides an intriguing insight into the immunometabolic pressures that support pathogen immune evasion adaptive mechanisms, akin to antibiotic selective pressure.

S. aureus small colony variants

The subversion of host immunometabolism by pathogens profoundly influences the infection outcome, as highlighted in the previous section. The compounding effect of accumulated metabolites on the epigenetic landscape of immune cells adds a further level of complexity to host–pathogen interactions. This is best exemplified by trained immunity or 'innate immune memory', which refers to increased

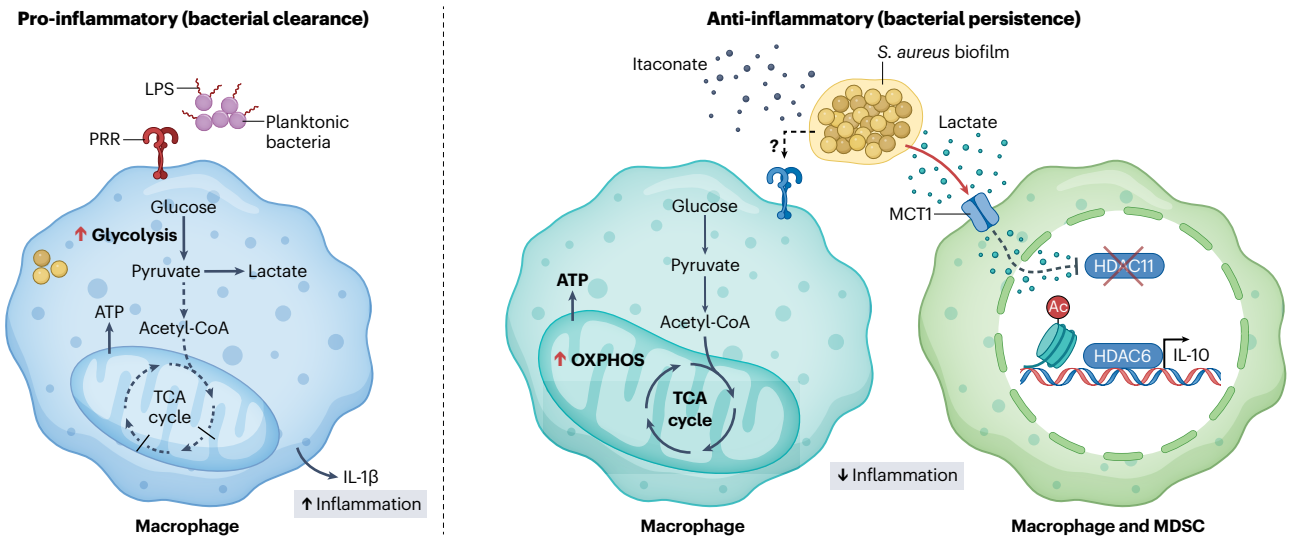


Fig. 3 | *Staphylococcus aureus* biofilm shapes the phenotype of leukocytes. The left panel shows metabolic reprogramming, characterized by increased glycolysis, in macrophages infected with live planktonic bacteria or stimulated with pathogen-associated molecular patterns such as lipopolysaccharide (LPS) recognized by pathogen recognition receptor (PRR). This skews the macrophage towards a pro-inflammatory phenotype (that is, the production of the pro-inflammatory cytokine IL-1β), which promotes bacterial clearance. The right panel shows two different strategies through which staphylococcal biofilms evade innate immunity. These include either rewiring host metabolism or epigenetic changes. *S. aureus* biofilm, which is induced by host-derived itaconate, promotes mitochondrial oxidative phosphorylation (OXPHOS) in macrophages and an anti-inflammatory phenotype. The mechanism remains unknown (indicated by the question mark). Lactate produced by *S. aureus* biofilms is transported into macrophages and myeloid-derived suppressor cells (MDSCs) through the monocarboxylate transporter 1 (MCT1), inhibits histone deacetylase 11 (HDAC11) and promotes IL-10 production. Ac, acetylation; CoA, coenzyme A; TCA, tricarboxylic acid.

Glossary

Accessory genome
Genes usually associated with mobile genetic elements that are present in only a subset of *S. aureus* strains. The accessory genome is one cause of variability in strain behaviour.

Biofilms
A sessile microbial community usually enclosed by a protective extracellular matrix and attached to a surface or other cells.

Bottlenecks
When a population (for example, bacterial population) is significantly reduced in size, limiting genetic diversity.

Carbon catabolite repression
(CCR). A bacterial global regulatory process that results in the selective use of substrates from a mixture of carbon sources.

Chronic granulomatous diseases
Rare X-linked recessive inherited immune deficiencies caused by defects in the enzyme, NADPH oxidase resulting in phagocytic dysfunction.

Colonization
The presence of *S. aureus* on a body site such as the skin, gut or anterior nares, without causing disease.

Core genome
Represents genes that are present in all *S. aureus* strains.

Efferocytosis
A process of phagocytic engulfment of dead or dying cells.

Evasion
Strategies used by bacteria to evade killing by the immune system.

Genetic drift
A change in the frequency of an existing genetic variant in a population owing to random chance.

Genome-wide association studies
The use of statistical genomics methods to identify the genetic variants linked to a particular phenotype.

Insertion sequences
A short segment of DNA that can move within the *S. aureus* chromosome as a simple transposable element and contribute to bacterial adaptation.

Invasion
The ability of a bacterial pathogen to spread to other locations in the host by invading host cells, such as the transition from the anterior nares (colonization) to the bloodstream (invasion).

Leukotoxins
Toxin proteins that penetrate lipid bilayers to form pores.

Lipopolysaccharide
Important outer membrane component of Gram-negative bacterial cell walls that acts as an endotoxin.

Machine learning
The application of computer systems using statistical models and algorithms to draw conclusions from data.

Microbial surface components recognizing adhesive matrix molecules
(MSCRAMMs). Adhesin proteins that are important in the initial binding of *S. aureus* to host tissues.

Mobile genetic elements
Sequences of genetic material that can change places in the *S. aureus* chromosome or move between bacterial chromosomes.

Persistence
Broadly refers to the ability of bacterial cells, including *S. aureus*, to cause persistent infection, despite the activity of the immune system or antibiotic therapy.

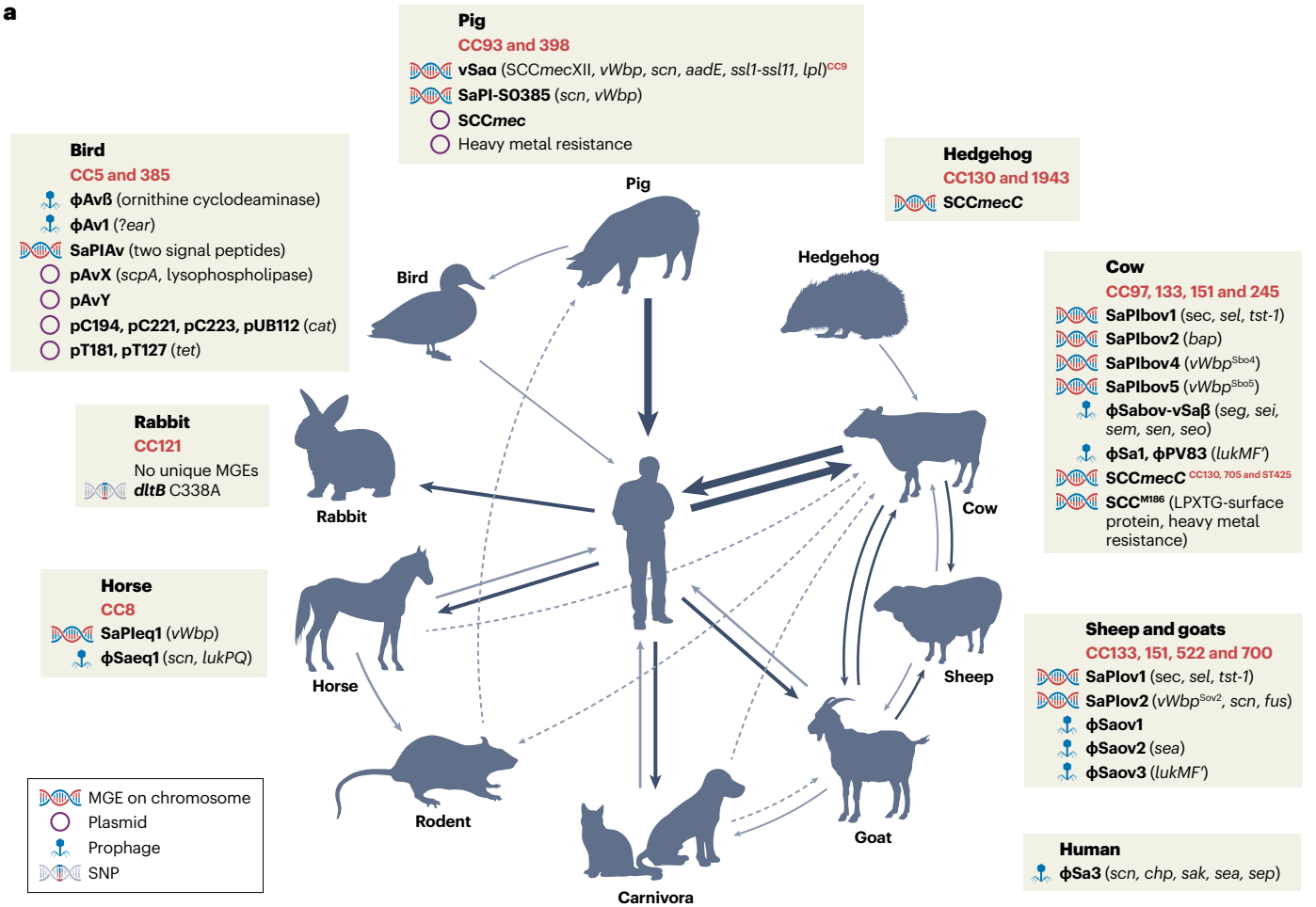
Pyomyositis
Deep infection in the skeletal muscles, usually associated with abscess formation.

Recurrent
The propensity for *S. aureus* infection to recur after initial successful therapy through surgery and/or antibiotics.

Small colony variant
(SCV). A slow-growing (small colony) population of *S. aureus* that is associated with persistent and recurrent infections.

innate immune protection against a secondary challenge following primary infection. Trained macrophages undergo substantial metabolic changes including the hallmark increase in glycolysis, and they accumulate metabolites that induce epigenetic rewiring of cellular function[95]. Accumulated fumarate inhibits KDM5 histone demethylases, which promotes trimethylation at the fourth lysine residue of the histone protein H3 (H3K4me3) at the promoters of pro-inflammatory cytokine genes such as those encoding tumour necrosis factor and IL-6, thus inducing their rapid production on restimulation and secondary infection.

In a mouse skin infection model, priming by previous infection with wild type *S. aureus* USA300 isolate LAC reduced skin lesion severity and bacterial burden on secondary infection. This protection was highly localized and mediated by macrophages[96,97]. Importantly, adoptive transfer of wild type *S. aureus*-primed macrophages into naive mouse skin afforded protection in vivo. However, priming by an *S. aureus* SCV prototype, Δ*hemB*, or a host-adapted isolate from a patient with atopic dermatitis did not confer protection from secondary infection[98,99]. SCVs, which are often associated with chronic infections, were first characterized in 1995 as phenotypically distinct from normal *S. aureus* colonies[100]. SCVs are slow-growing, have pinpoint colony size and may carry mutations in genes associated with electron transport chain components such as menadione and haeme[22]. As a result of these mutations, SCVs often have altered bacterial metabolism with decreased TCA cycle or OXPHOS activity and increased glycolysis to meet their energy requirements[101]. The ability of SCVs to abrogate trained immunity and cause recurrent infection was mediated by increased expression of *fumC*, which encodes the enzyme fumarate hydratase to degrade fumarate[99]. Increased *fumC* expression was also observed in *S. aureus* isolates from patients with atopic dermatitis and cystic fibrosis[98,102]. The blunting of immunity by host-adapted *S. aureus* isolates remains to be explored in the persistently colonized organs, such as the lungs of patients with cystic fibrosis. Genomics-enabled studies are providing further insights into SCV genetic mechanisms such as chromosomal rearrangements[103]. Wider uptake of such approaches is likely to accelerate the discovery of other bacterial genomic modifications occurring in SCVs and arising in response to immune selective pressures and promoting persistent infections (Supplementary Box 2 and Supplementary Fig. 2).



S. aureus host specificity and adaptation

Understanding host adaptation is providing insights into host-specific immune evasion mechanisms and by extension enhances our understanding of disease at the species level. It is well recognized that *S. aureus* is a multispecies pathogen, able to cause disease in humans and livestock. The diseases in livestock include mastitis in cows, goats, sheep and rabbits, skin infections in pigs and rabbits and invasive infections in chickens[104]. Advances in agricultural practices are increasing transmission risks between humans and livestock. For instance, the threat to human health from livestock-associated *S. aureus* has been well described for the pig-associated clones, especially CC398 (ref. [105]) as well as ST9, ST5 (ref. [106]) and ST93 (ref. [107]); however, key immune evasion factors (*scn*, *chp*, *sak*, *sea*, which are encoded by the immune evasion complex) are observed in isolates from humans but not from pigs. Other clones from livestock can also spread in humans[108] and exemplified by the recent description of the emergence of MRSA in

Fig. 4 | *Staphylococcus aureus* host species adaptation over time. a, Host-switching modelling of *Staphylococcus aureus* between species identified humans as a major transmission hub[104]. Overlayed are reported host-specific mobile genetic elements (MGEs) in pigs[118,119], cows[119,120], small ruminants[121,122], horses[123], birds[119] and hedgehogs[109]; and their major associated clonal complexes (CCs). MGEs belonging to the non-dominant *S. aureus* lineages in pigs and cows have been reported, these CCs and sequence types (STs) are denoted in superscript. *S. aureus* infecting rabbits has no unique MGEs. A single nucleotide polymorphism (C338A) in chromosomal gene, *dtlB*, causes a non-synonymous mutation required for infection of rabbits by CC121 (see Supplementary Fig. 3c for all CC121 rabbit adaptations). No unique *S. aureus* MGEs have been described in rodents or carnivora. Globally disseminated, multihost lineage CC398 has been reported in rodents and carnivora, but no CCs are recognized as dominant in these species (see Supplementary Fig. 3d for the mechanism of broad host distribution for CC398). Human *S. aureus* strains possess the ϕSa3 prophage (β-haemolysin-converting phage) encoding a human-specific evasion cluster that integrates into *hlb*, causing loss of β-toxin production. Many animal-adapted *S. aureus* strains have lost ϕSa3, resulting in restored β-toxin production and increased fitness. Linewidth represents frequency of host jumps. **b**, Function of *S. aureus* genes carried on animal-adapted MGEs. *S. aureus* superantigens are virulence factors that cause nonspecific T cell activation and massive cytokine release. This excessive activation of the host immune system paradoxically promotes infection; antibiotic resistance is conferred by multiple resistance determinants; species-adapted leukodicins, encoded by *luk*, cause host neutrophil lysis. von Willebrand-binding protein (vWbp) causes host-specific plasma coagulation; adhesion protein (Bap) promotes *S. aureus* adhesion to bovine mammary mucosa and biofilm production resulting in colonization, contributing to the pathogenesis of bovine mastitis. Putative adhesin, LPXTG-surface protein promotes colonization (see Supplementary Fig. 3a for bovine-specific *S. aureus* adaptions); staphylococcal complement inhibitor (SCIN) encoded by *scn* blocks the activation of the host complement system; Avian-specific *scpA* encodes the thiol protease staphopain A, which is associated with enhanced pathogenicity through the inhibition of host neutrophil activation and chemotaxis (see Supplementary Fig. 3b for *S. aureus* CC5 adaptations in chickens). [#]Putative adhesin. ϕSabov-vSa, bacteriophage ϕ-*S. aureus* bovine in genomic island v-*S. aureus*; LPXTG, conserved peptide motif in surface linked proteins cleaved between the threonine and glycine residues and covalently attached to cell wall peptidoglycan; SCC*mecC*, staphylococcal cassette chromosome methicillin C resistance gene; TSST-1, toxic shock syndrome toxin-1; vSaα, genomic island v-*S. aureus*-α.

non-human mammals, in this case European hedgehogs, which subsequently spread to livestock and humans, before the use of methicillin as an antibiotic[109].

Host adaptation can be shaped by genetic drift (a process of neutral diversification) or adaptive evolution, whereby beneficial mutations are selected or deleterious mutations removed in the new host[110]. Genetic drift is typically associated with reduction of the effective population size as it occurs in 'bottlenecks'[111]. For example, in the transition from colonizing to invasive pathogen, only a few cells of a more diverse *S. aureus* colonizing population survive within a macrophage or other phagocytic cell (bottlenecks) and then subsequently expand to cause invasive disease[112]. By contrast, adaptive evolution results in an increase in frequency of the beneficial variant. It is likely that both have a role during infection and adaptation[113]. Combining comparative population genomics including both human and animal isolates with functional genomic studies will illuminate the staphylococcal drivers of host adaptation and determine the host specific factors that support bacterial survival[114]. Such information will guide the design of strategies that prevent the emergence of new pandemic clones and cross-species transmission.

Over the past decade, genomics studies have provided a comprehensive mapping of *S. aureus* accessory genome elements that are specific to certain host species and enriched the understanding of *S. aureus* host adaptation[115], revealing that immune evasion factors targeted to certain hosts were specifically produced by host-adapted *S. aureus* lineages. Larger scale analyses of *S. aureus* population genomics across multiple host species and humans demonstrated that the latter were the major donors for *S. aureus* host-switching events, and that cows represent a potential reservoir of new epidemic clones in humans[104]. Understanding the gene flow in different ecological niches highlights host-specific gene repertoires that are linked to host adaptation, with *S. aureus* accessory genome elements from isolates from the same host species highly correlated even across diverse *S. aureus* clonal complexes[104]. By contrast, core genome evolution can be traced through the assessment of adaptive mutations that result from diversifying selection, with some biological pathways found to be under positive selection in humans (Fig. 4 and Supplementary Fig. 3). Antimicrobial resistance elements are another component of the accessory genome which can reveal unique host species patterns. Unsurprisingly, human isolates are enriched for elements that provide resistance to antimicrobials used in humans such as β-lactams, whereas tetracycline and antiseptic resistance are significantly associated with pig isolates[104].

The exploration of host-specific adaptation in experimental models could provide robust validation of genetic signatures that have been identified by population genomics analyses. An exemplar of a targeted approach is the study of ST121 *S. aureus*, which causes serious infections in both human and rabbit hosts. Bayesian modelling of genomic data suggests a human-to-rabbit host jump about 50 years ago, with genome diversification between host species since then[116]. Although no specific mobile elements were identified in the genome of rabbit-associated *S. aureus* ST121, the functional interrogation of mutations between host strains identified a single point mutation in the *dltB* gene. Such mutation was sufficient to cause the rabbit disease phenotype in animal models[116]; however, the mechanisms by which *dltB* mutations result in this host adaptation are yet to be defined (Fig. 4 and Supplementary Fig. 3).

The ability of *S. aureus* to adapt to new hosts, and the capacity to adapt to population bottlenecks, has been further explored in a model of human-to-ovine host switching complemented by large-scale genomic analyses[117]. This revealed that *S. aureus* could overcome substantial population bottlenecks and acquire beneficial mutations over time. However, there was not a strong signal of convergence, which suggests that multiple pathways to adaptation were occurring in this model. The exploitation of host-switching models will further enhance the understanding of adaptive mechanisms underlying species-specific pathogenesis.

Conclusions and outlook

S. aureus remains a formidable multispecies pathogen and major challenge to human health. Fuelled by the major progress in omics, important advances have been made in the past 10 years to understand the duality of *S. aureus* as an asymptomatic colonizer and devastating invasive pathogen. Although new immune evasion molecules and novel ligands for well-described molecules have been identified, a holistic

understanding of the in vivo dynamics and interactions within host will be unlikely revealed by a traditional one-by-one gene discovery approach. The variability in the immune evasion repertoire across *S. aureus* clones and the heterogeneity of host responses substantially complicate this issue, particularly in complex clinical contexts. Thus, systems-level experimental approaches that account for *S. aureus* strain variation, combined with improved laboratory infection models (perhaps even controlled human infection or colonization models) that consider immune response and microbiome context, are needed to better understand the roles of critical *S. aureus* factors during infection. Such advances could help address the issues that have challenged *S. aureus* vaccine development such as immune imprinting resulting from previous *S. aureus* exposure.

With its capacity to switch between host species, transition from colonization to invasion, or developing antibiotic resistance and persistence, *S. aureus* has the propensity to adapt to new environments. An integrated understanding of how *S. aureus* genomics, evolution and adaptation intersect with virulence and immune evasion strategies will guide the design of prevention strategies and therapeutic approaches. Other potential avenues could stem from the identification of human genetic signatures associated with *S. aureus* disease risk as well as from the microbiome composition of the skin, the nose and the gut.

It is also becoming increasingly apparent that immunometabolism has a key role in the pathogenesis of *S. aureus* infections. However, microbial metabolism has often been neglected, with many research groups instead using pathogen-associated molecular patterns as a proxy for bacterial stimulation. There is increasing evidence that staphylococcal metabolic flexibility is not only critical for bacterial persistence during infection but also in shaping the host immunometabolic response. Importantly, this metabolic interplay between host and *S. aureus* varies by infection site. Targeting immunometabolism as an alternative or complementary therapeutic strategy to enhance multidrug-resistant *S. aureus* clearance will become more realistic as our understanding of host–pathogen metabolic interactions deepens.

Ultimately, advancing the study of *S. aureus* host–pathogen interactions by acknowledging the roles of other microbiota and immunometabolism, and further understanding pathogen genetic variability and adaptation will generate holistic insights that lead to meaningful clinical impact on diseases caused by *S. aureus*.

Published online: 27 January 2023

References

1. Lowy, F. D. *Staphylococcus aureus* infections. *N. Engl. J. Med.* **339**, 520–532 (1998).
2. Wertheim, H. F. et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* **5**, 751–762 (2005).
3. Williams, R. E. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol. Rev.* **27**, 56–71 (1963).
4. Krismer, B., Weidenmaier, C., Zipperer, A. & Peschel, A. The commensal lifestyle of *Staphylococcus aureus* and its interactions with the nasal microbiota. *Nat. Rev. Microbiol.* **15**, 675–687 (2017).
   Excellent review on the nasal microbiome and the interaction with *S. aureus*.
5. Clarridge, J. E. III, Harrington, A. T., Roberts, M. C., Soge, O. O. & Maquelin, K. Impact of strain typing methods on assessment of relationship between paired nares and wound isolates of methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* **51**, 224–231 (2013).
6. Raineri, E. J. M., Altulea, D. & van Dijl, J. M. Staphylococcal trafficking and infection-from 'nose to gut' and back. *FEMS Microbiol. Rev.* **46**, fuab041 (2022).
7. Spaan, A. N., van Strijp, J. A. G. & Torres, V. J. Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. *Nat. Rev. Microbiol.* **15**, 435–447 (2017).
   Comprehensive review of the staphylococcal leukocidins.
8. Thammavongsa, V., Kim, H. K., Missiakas, D. & Schneewind, O. Staphylococcal manipulation of host immune responses. *Nat. Rev. Microbiol.* **13**, 529–543 (2015).
9. Montgomery, C. P., David, M. Z. & Daum, R. S. Host factors that contribute to recurrent staphylococcal skin infection. *Curr. Opin. Infect. Dis.* **28**, 253–258 (2015).
10. Kallen, A. J. et al. Health care-associated invasive MRSA infections, 2005–2008. *JAMA* **304**, 641–648 (2010).
11. Jansen, K. U., Girgenti, D. Q., Scully, I. L. & Anderson, A. S. Vaccine review: '*Staphylococcus aureus* vaccines: problems and prospects'. *Vaccine* **31**, 2723–2730 (2013).
12. Wollenberg, M. S. et al. Propionibacterium-produced coproporphyrin III induces *Staphylococcus aureus* aggregation and biofilm formation. *mBio* **5**, e01286-14 (2014).
13. Costello, E. K. et al. Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694–1697 (2009).
14. Torres Salazar, B. O., Heilbronner, S., Peschel, A. & Krismer, B. Secondary metabolites governing microbiome interaction of Staphylococcal pathogens and commensals. *Micro. Physiol.* **31**, 198–216 (2021).
15. Lister, J. L. & Horswill, A. R. *Staphylococcus aureus* biofilms: recent developments in biofilm dispersal. *Front. Cell Infect. Microbiol.* **4**, 178 (2014).
16. Archer, N. K. et al. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. *Virulence* **2**, 445–459 (2011).
17. Bhattacharya, M. et al. *Staphylococcus aureus* biofilms release leukocidins to elicit extracellular trap formation and evade neutrophil-mediated killing. *Proc. Natl Acad. Sci. USA* **115**, 7416–7421 (2018).
18. Chambers, H. F. & Deleo, F. R. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat. Rev. Microbiol.* **7**, 629–641 (2009).
19. Turner, N. A. et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat. Rev. Microbiol.* **17**, 203–218 (2019).
20. Howden, B. P., Davies, J. K., Johnson, P. D., Stinear, T. P. & Grayson, M. L. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin. Microbiol. Rev.* **23**, 99–139 (2010).
21. Gao, W. et al. The RpoB H481Y rifampicin resistance mutation and an active stringent response reduce virulence and increase resistance to innate immune responses in *Staphylococcus aureus*. *J. Infect. Dis.* **207**, 929–939 (2013).
22. Proctor, R. A. et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat. Rev. Microbiol.* **4**, 295–305 (2006).
23. Moller, A. G., Petit, R. A. III & Read, T. D. Species-scale genomic analysis of *Staphylococcus aureus* genes influencing phage host range and their relationships to virulence and antibiotic resistance genes. *mSystems* **7**, e0108321 (2022).
24. Howden, B. P. et al. Evolution of multidrug resistance during *Staphylococcus aureus* infection involves mutation of the essential two component regulator WalKR. *PLoS Pathog.* **7**, e1002359 (2011).
25. Mwangi, M. M. et al. Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. *Proc. Natl Acad. Sci. USA* **104**, 9451–9456 (2007).
26. Gao, W. et al. Large tandem chromosome expansions facilitate niche adaptation during persistent infection with drug-resistant *Staphylococcus aureus*. *Microb. Genom.* **1**, e000026 (2015).
27. Panton, P. N. & Valentine, F. C. O. Staphylococcal toxin. *Lancet* **219**, 506–508 (1932).
28. Cheung, G. Y. C., Bae, J. S. & Otto, M. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence* **12**, 547–569 (2021).
29. Curnutte, J. T., Whitten, D. M. & Babior, B. M. Defective superoxide production by granulocytes from patients with chronic granulomatous disease. *N. Engl. J. Med.* **290**, 593–597 (1974).
30. Spaan, A. N., Surewaard, B. G., Nijland, R. & van Strijp, J. A. Neutrophils versus *Staphylococcus aureus*: a biological tug of war. *Annu. Rev. Microbiol.* **67**, 629–650 (2013).
31. Stapels, D. A. et al. *Staphylococcus aureus* secretes a unique class of neutrophil serine protease inhibitors. *Proc. Natl Acad. Sci. USA* **111**, 13187–13192 (2014).
32. Kretschmer, D. et al. *Staphylococcus aureus* depends on eap proteins for preventing degradation of its phenol-soluble modulin toxins by neutrophil serine proteases. *Front. Immunol.* **12**, 701093 (2021).
33. Stapels, D. A. et al. *Staphylococcus aureus* protects its immune-evasion proteins against degradation by neutrophil serine proteases. *Cell Microbiol.* **18**, 536–545 (2016).
34. Ploscariu, N. T., de Jong, N. W. M., van Kessel, K. P. M., van Strijp, J. A. G. & Geisbrecht, B. V. Identification and structural characterization of a novel myeloperoxidase inhibitor from *Staphylococcus delphini*. *Arch. Biochem. Biophys.* **645**, 1–11 (2018).
35. de Jong, N. W. M. et al. Immune evasion by a staphylococcal inhibitor of myeloperoxidase. *Proc. Natl Acad. Sci. USA* **114**, 9439–9444 (2017).
36. Loffler, B. et al. *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PloS Pathog.* **6**, e1000715 (2010).
37. Vrieling, M. et al. Population analysis of *Staphylococcus aureus* reveals a cryptic, highly prevalent superantigen SelW that contributes to the pathogenesis of bacteremia. *mBio* **11**, e02082-20 (2020).
38. Wilson, G. J. et al. A novel core genome-encoded superantigen contributes to lethality of community-associated MRSA necrotizing pneumonia. *PloS Pathog.* **7**, e1002271 (2011).
39. Tuffs, S. W. et al. The *Staphylococcus aureus* superantigen SelX is a bifunctional toxin that inhibits neutrophil function. *PloS Pathog.* **13**, e1006461 (2017).
40. Foster, T. J., Geoghegan, J. A., Ganesh, V. K. & Hook, M. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.* **12**, 49–62 (2014).

41. Corrigan, R. M., Miajlovic, H. & Foster, T. J. Surface proteins that promote adherence of Staphylococcus aureus to human desquamated nasal epithelial cells. *BMC Microbiol.* **9**, 22 (2009).
42. Cheng, A. G. et al. Genetic requirements for *Staphylococcus aureus* abscess formation and persistence in host tissues. *FASEB J.* **23**, 3393–3404 (2009).
43. Askarian, F. et al. Serine-aspartate repeat protein D increases *Staphylococcus aureus* virulence and survival in blood. *Infect. Immun.* **85**, e00559-16 (2017).
44. Zhang, Y. et al. *Staphylococcus aureus* SdrE captures complement factor H's C-terminus via a novel 'close, dock, lock and latch' mechanism for complement evasion. *Biochem. J.* **474**, 1619–1631 (2017).
45. Speziale, P. & Pietrocola, G. The multivalent role of fibronectin-binding proteins A and B (FnBPA and FnBPB) of *Staphylococcus aureus* in host infections. *Front. Microbiol.* **11**, 2054 (2020).
46. Pietrocola, G. et al. Fibronectin-binding protein B (FnBPB) from *Staphylococcus aureus* protects against the antimicrobial activity of histones. *J. Biol. Chem.* **294**, 3588–3602 (2019).
47. Thammavongsa, V., Missiakas, D. M. & Schneewind, O. *Staphylococcus aureus* degrades neutrophil extracellular traps to promote immune cell death. *Science* **342**, 863–866 (2013).
48. Soh, K. Y., Loh, J. M. S. & Proft, T. Cell wall-anchored 5′-nucleotidases in Gram-positive cocci. *Mol. Microbiol.* **113**, 691–698 (2020).
49. Berends, E. T. M. et al. *Staphylococcus aureus* impairs the function of and kills human dendritic cells via the LukAB toxin. *mBio* **10**, e01918-18 (2019).
50. Badarau, A., Trstenjak, N. & Nagy, E. Structure and function of the two-component cytotoxins of *Staphylococcus aureus* — learnings for designing novel therapeutics. *Adv. Exp. Med. Biol.* **966**, 15–35 (2017).
51. Perelman, S. S. et al. Genetic variation of staphylococcal LukAB toxin determines receptor tropism. *Nat. Microbiol.* **6**, 731–745 (2021).
52. Spaan, A. N. et al. The staphylococcal toxin Panton-Valentine leukocidin targets human C5a receptors. *Cell Host Microbe* **13**, 584–594 (2013).
53. Haapasalo, K. et al. *Staphylococcus aureus* toxin LukSF dissociates from its membrane receptor target to enable renewed ligand sequestration. *FASEB J.* **33**, 3807–3824 (2019).
54. Tromp, A. T. et al. Human CD45 is an F-component-specific receptor for the staphylococcal toxin Panton-Valentine leukocidin. *Nat. Microbiol.* **3**, 708–717 (2018).
55. Knop, J. et al. *Staphylococcus aureus* infection in humanized mice: a new model to study pathogenicity associated with human immune response. *J. Infect. Dis.* **212**, 435–444 (2015).
56. Prince, A., Wang, H., Kitur, K. & Parker, D. Humanized mice exhibit increased susceptibility to *Staphylococcus aureus* pneumonia. *J. Infect. Dis.* **215**, 1386–1395 (2017).
57. Tseng, C. W. et al. Increased susceptibility of humanized NSG mice to Panton-Valentine leukocidin and *Staphylococcus aureus* skin infection. *PLoS Pathog.* **11**, e1005292 (2015).
58. Muthukrishnan, G. et al. Humanized mice exhibit exacerbated abscess formation and osteolysis during the establishment of implant-associated *Staphylococcus aureus* osteomyelitis. *Front. Immunol.* **12**, 651515 (2021).
59. Chow, S. H. et al. Targeting NLRP3 and staphylococcal pore-forming toxin receptors in human-induced pluripotent stem cell-derived macrophages. *J. Leukoc. Biol.* **108**, 967–981 (2020).
60. Lees, J. A. & Bentley, S. D. Bacterial GWAS: not just gilding the lily. *Nat. Rev. Microbiol.* **14**, 406 (2016).
61. Young, B. C. et al. Panton-Valentine leucocidin is the key determinant of *Staphylococcus aureus* pyomyositis in a bacterial GWAS. *eLife* **8**, e42486 (2019).
Statistical genomics study linking a *S. aureus* virulence factor to a clinical manifestation.
62. Koymans, K. J. et al. Staphylococcal superantigen-like protein 1 and 5 (SSL1 & SSL5) limit neutrophil chemotaxis and migration through MMP-inhibition. *Int. J. Mol. Sci.* **17**, 1072 (2016).
63. Bestebroer, J. et al. Staphylococcal SSL5 inhibits leukocyte activation by chemokines and anaphylatoxins. *Blood* **113**, 328–337 (2009).
64. Tang, A. et al. *Staphylococcus aureus* superantigen-like protein SSL1: a toxic protease. *Pathogens* **8**, 2 (2019).
65. Koymans, K. J. et al. The TLR2 antagonist Staphylococcal superantigen-like protein 3 acts as a virulence factor to promote bacterial pathogenicity in vivo. *J. Innate Immun.* **9**, 561–573 (2017).
66. Zhao, Y. et al. Staphylococcal superantigen-like protein 13 activates neutrophils via formyl peptide receptor 2. *Cell Microbiol.* **20**, e12941 (2018).
67. Diebolder, C. A. et al. Complement is activated by IgG hexamers assembled at the cell surface. *Science* **343**, 1260–1263 (2014).
68. Becker, S., Frankel, M. B., Schneewind, O. & Missiakas, D. Release of protein A from the cell wall of *Staphylococcus aureus*. *Proc. Natl Acad. Sci. USA* **111**, 1574–1579 (2014).
69. O'Halloran, D. P., Wynne, K. & Geoghegan, J. A. Protein A is released into the *Staphylococcus aureus* culture supernatant with an unprocessed sorting signal. *Infect. Immun.* **83**, 1598–1609 (2015).
70. Falugi, F., Kim, H. K., Missiakas, D. M. & Schneewind, O. Role of protein A in the evasion of host adaptive immune responses by *Staphylococcus aureus*. *mBio* **4**, e00575-13 (2013).
71. Cruz, A. R. et al. Staphylococcal protein A inhibits complement activation by interfering with IgG hexamer formation. *Proc. Natl Acad. Sci. USA* **118**, e2016772118 (2021).
72. Hong, X. et al. Staphylococcal protein A promotes colonization and immune evasion of the epidemic healthcare-associated MRSA ST239. *Front. Microbiol.* **7**, 951 (2016).
73. Smith, E. J. et al. The immune evasion protein Sbi of *Staphylococcus aureus* occurs both extracellularly and anchored to the cell envelope by binding lipoteichoic acid. *Mol. Microbiol.* **83**, 789–804 (2012).
74. Dunphy, R. W. et al. Staphylococcal complement evasion protein Sbi stabilises C3d dimers by inducing an N-terminal helix swap. *Front. Immunol.* **13**, 892234 (2022).
75. Dasari, P. et al. The protease SplB of *Staphylococcus aureus* targets host complement components and inhibits complement-mediated bacterial opsonophagocytosis. *J. Bacteriol.* **204**, e0018421 (2022).
76. Bhattacharya, M. et al. Leukocidins and the nuclease nuc prevent neutrophil-mediated killing of *Staphylococcus aureus* biofilms. *Infect. Immun.* **88**, e00372-20 (2020).
77. Schilcher, K. & Horswill, A. R. Staphylococcal biofilm development: structure, regulation, and treatment strategies. *Microbiol. Mol. Biol. Rev.* **84**, e00026-19 (2020).
Comprehensive review of *S. aureus* biofilms.
78. Ricciardi, B. F. et al. *Staphylococcus aureus* evasion of host immunity in the setting of prosthetic joint infection: biofilm and beyond. *Curr. Rev. Musculoskelet. Med.* **11**, 389–400 (2018).
79. Arciola, C. R., Campoccia, D. & Montanaro, L. Implant infections: adhesion, biofilm formation and immune evasion. *Nat. Rev. Microbiol.* **16**, 397–409 (2018).
80. Heim, C. E., Vidlak, D. & Kielian, T. Interleukin-10 production by myeloid-derived suppressor cells contributes to bacterial persistence during *Staphylococcus aureus* orthopedic biofilm infection. *J. Leukoc. Biol.* **98**, 1003–1013 (2015).
81. Heim, C. E. et al. IL-12 promotes myeloid-derived suppressor cell recruitment and bacterial persistence during *Staphylococcus aureus* orthopedic implant infection. *J. Immunol.* **194**, 3861–3872 (2015).
82. Heim, C. E. et al. Myeloid-derived suppressor cells contribute to *Staphylococcus aureus* orthopedic biofilm infection. *J. Immunol.* **192**, 3778–3792 (2014).
83. Heim, C. E., West, S. C., Ali, H. & Kielian, T. Heterogeneity of Ly6G(+) Ly6C(+) myeloid-derived suppressor cell infiltrates during *Staphylococcus aureus* biofilm infection. *Infect. Immun.* **86**, e00684-18 (2018).
84. Yamada, K. J. et al. Monocyte metabolic reprogramming promotes pro-inflammatory activity and *Staphylococcus aureus* biofilm clearance. *PLoS Pathog.* **16**, e1008354 (2020).
85. Gabrilovich, D. I. & Nagaraj, S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* **9**, 162–174 (2009).
86. O'Neill, L. A., Kishton, R. J. & Rathmell, J. A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* **16**, 553–565 (2016).
87. Tannahill, G. M. et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* **496**, 238–242 (2013).
88. Lampropoulou, V. et al. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab.* **24**, 158–166 (2016).
89. Hooftman, A. et al. The immunomodulatory metabolite itaconate modifies NLRP3 and inhibits inflammasome activation. *Cell Metab.* **32**, 468–478.e7 (2020).
90. Qin, W. et al. S-glycosylation-based cysteine profiling reveals regulation of glycolysis by itaconate. *Nat. Chem. Biol.* **15**, 983–991 (2019).
91. Heim, C. E. et al. Lactate production by *Staphylococcus aureus* biofilm inhibits HDAC11 to reprogramme the host immune response during persistent infection. *Nat. Microbiol.* **5**, 1271–1284 (2020).
Sophisticated host–pathogen study using at-scale transposon mutagenesis to reveal the role mechanism of *S. aureus* lactate in regulation of IL-10 expression.
92. Tomlinson, K. L. & Riquelme, S. A. Host–bacteria metabolic crosstalk drives *S. aureus* biofilm. *Microb. Cell* **8**, 106–107 (2021).
93. Tomlinson, K. L. et al. *Staphylococcus aureus* induces an itaconate-dominated immunometabolic response that drives biofilm formation. *Nat. Commun.* **12**, 1399 (2021).
94. Michelucci, A. et al. Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc. Natl Acad. Sci. USA* **110**, 7820–7825 (2013).
95. Arts, R. J. et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab.* **24**, 807–819 (2016).
96. Chan, L. C. et al. Innate immune memory contributes to host defense against recurrent skin and skin structure infections caused by methicillin-resistant *Staphylococcus aureus*. *Infect. Immun.* **85**, e00876-16 (2017).
97. Chan, L. C. et al. Protective immunity in recurrent *Staphylococcus aureus* infection reflects localized immune signatures and macrophage-conferred memory. *Proc. Natl Acad. Sci. USA* **115**, E11111–E11119 (2018).
98. Acker, K. P. et al. Strains of *Staphylococcus aureus* that colonize and infect skin harbor mutations in metabolic genes. *iScience* **19**, 281–290 (2019).
99. Wong Fok Lung, T. et al. *Staphylococcus aureus* small colony variants impair host immunity by activating host cell glycolysis and inducing necroptosis. *Nat. Microbiol.* **5**, 141–153 (2020).
100. Proctor, R. A., van Langevelde, P., Kristjansson, M., Maslow, J. N. & Arbeit, R. D. Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. *Clin. Infect. Dis.* **20**, 95–102 (1995).
101. Kriegeskorte, A. et al. *Staphylococcus aureus* small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. *Front. Cell Infect. Microbiol.* **4**, 141 (2014).
102. Gabryszewski, S. J. et al. Metabolic adaptation in methicillin-resistant *Staphylococcus aureus* pneumonia. *Am. J. Respir. Cell Mol. Biol.* **61**, 185–197 (2019).
103. Guerillot, R. et al. Unstable chromosome rearrangements in *Staphylococcus aureus* cause phenotype switching associated with persistent infections. *Proc. Natl Acad. Sci. USA* **116**, 20135–20140 (2019).
Identification of a reversible genome rearrangement contributing to the SCV phenotype in *S. aureus*.

104. Richardson, E. J. et al. Gene exchange drives the ecological success of a multi-host bacterial pathogen. *Nat. Ecol. Evol.* **2**, 1468–1478 (2018).
Excellent demonstration of bacterial population genomics to reveal the extent to which humans are shaping the evolution of *S. aureus* and exchange between different animal hosts.
105. Price, L. B. et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *mBio* **3**, e00305-11 (2012).
106. Hau, S. J., Sun, J., Davies, P. R., Frana, T. S. & Nicholson, T. L. Comparative prevalence of immune evasion complex genes associated with beta-hemolysin converting bacteriophages in MRSA ST5 isolates from swine, swine facilities, humans with swine contact, and humans with no swine contact. *PLoS ONE* **10**, e0142832 (2015).
107. Sahibzada, S. et al. Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. *Sci. Rep.* **7**, 5273 (2017).
108. Spoor, L. E. et al. Livestock origin for a human pandemic clone of community-associated methicillin-resistant *Staphylococcus aureus*. *mBio* **4**, e00356-13 (2013).
109. Larsen, J. et al. Emergence of methicillin resistance predates the clinical use of antibiotics. *Nature* **602**, 135–141 (2022).
Large-scale genomic study identifying an animal source for antimicrobial-resistant *S. aureus* in humans.
110. Hallatschek, O., Hersen, P., Ramanathan, S. & Nelson, D. R. Genetic drift at expanding frontiers promotes gene segregation. *Proc. Natl Acad. Sci. USA* **104**, 19926–19930 (2007).
111. Nei, M. Selectionism and neutralism in molecular evolution. *Mol. Biol. Evol.* **22**, 2318–2342 (2005).
112. McVicker, G. et al. Clonal expansion during *Staphylococcus aureus* infection dynamics reveals the effect of antibiotic intervention. *PLoS Pathog.* **10**, e1003959 (2014).
113. Didelot, X., Walker, A. S., Peto, T. E., Crook, D. W. & Wilson, D. J. Within-host evolution of bacterial pathogens. *Nat. Rev. Microbiol.* **14**, 150–162 (2016).
114. Sheppard, S. K., Guttman, D. S. & Fitzgerald, J. R. Population genomics of bacterial host adaptation. *Nat. Rev. Genet.* **19**, 549–565 (2018).
Comprehensive review of bacterial host species adaptation.
115. Guinane, C. M. et al. Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. *Genome Biol. Evol.* **2**, 454–466 (2010).
116. Viana, D. et al. A single natural nucleotide mutation alters bacterial pathogen host tropism. *Nat. Genet.* **47**, 361–366 (2015).
117. Bacigalupe, R., Tormo-Mas, M. A., Penades, J. R. & Fitzgerald, J. R. A multihost bacterial pathogen overcomes continuous population bottlenecks to adapt to new host species. *Sci. Adv.* **5**, eaax0063 (2019).
Experimental study demonstrating the capacity of *S. aureus* to acquire beneficial mutations alleviating evolutionary bottlenecks and enabling its adaptation to different hosts.
118. Zhou, W. et al. WGS analysis of ST9-MRSA-XII isolates from live pigs in China provides insights into transmission among porcine, human and bovine hosts. *J. Antimicrob. Chemother.* **73**, 2652–2661 (2018).
119. Haag, A. F., Fitzgerald, J. R. & Penades, J. R. *Staphylococcus aureus* in animals. *Microbiol. Spectr.* https://doi.org/10.1128/microbiolspec.GPP3-0060-2019 (2019).
120. Vrieling, M. et al. Bovine *Staphylococcus aureus* secretes the leukocidin LukMF′ to kill migrating neutrophils through CCR1. *mBio* **6**, e00335 (2015).
121. Matuszewska, M., Murray, G. G. R., Harrison, E. M., Holmes, M. A. & Weinert, L. A. The evolutionary genomics of host specificity in *Staphylococcus aureus*. *Trends Microbiol.* **28**, 465–477 (2020).
122. Park, S. & Ronholm, J. *Staphylococcus aureus* in agriculture: lessons in evolution from a multispecies pathogen. *Clin. Microbiol. Rev.* **34**, e00182-20 (2021).
123. de Jong, N. W. M. et al. Identification of a staphylococcal complement inhibitor with broad host specificity in equid *Staphylococcus aureus* strains. *J. Biol. Chem.* **293**, 4468–4477 (2018).
124. Petit, R. A. III & Read, T. D. Staphylococcus aureus viewed from the perspective of 40,000+ genomes. *Peer J.* **6**, e5261 (2018).
125. Lindsay, J. A. Genomic variation and evolution of *Staphylococcus aureus*. *Int. J. Med. Microbiol.* **300**, 98–103 (2010).
126. Malachowa, N. & DeLeo, F. R. Mobile genetic elements of *Staphylococcus aureus*. *Cell Mol. Life Sci.* **67**, 3057–3071 (2010).
127. Golubchik, T. et al. Within-host evolution of *Staphylococcus aureus* during asymptomatic carriage. *PLoS ONE* **8**, e61319 (2013).
128. Everitt, R. G. et al. Mobile elements drive recombination hotspots in the core genome of *Staphylococcus aureus*. *Nat. Commun.* **5**, 3956 (2014).
129. Prunier, A.-L. et al. High rate of macrolide resistance in *Staphylococcus aureus* strains from patients with cystic fibrosis reveals high proportions of hypermutable strains. *J. Infect. Dis.* **187**, 1709–1716 (2003).
130. Giulieri, S. G. et al. Niche-specific genome degradation and convergent evolution shaping *Staphylococcus aureus* adaptation during severe infections. *eLife* **11**, e77195 (2022).
Population genomics conducted on a very large clinical cohort to reveal with unprecedented resolution the bacterial genome-wide changes that are associated with the transition from colonizing to invasive *S. aureus* infections.
131. Giulieri, S. G. et al. Genomic exploration of sequential clinical isolates reveals a distinctive molecular signature of persistent *Staphylococcus aureus* bacteraemia. *Genome Med.* **10**, 65 (2018).
132. Klemm, E. J. et al. Emergence of host-adapted Salmonella *Enteritidis* through rapid evolution in an immunocompromised host. *Nat. Microbiol.* **1**, 15023 (2016).
133. Batut, B., Knibbe, C., Marais, G. & Daubin, V. Reductive genome evolution at both ends of the bacterial population size spectrum. *Nat. Rev. Microbiol.* **12**, 841–850 (2014).
134. Hall, M. D. et al. Improved characterisation of MRSA transmission using within-host bacterial sequence diversity. *eLife* **8**, 46402 (2019).
135. Long, D. R. et al. Polyclonality, shared strains, and convergent evolution in chronic CF *S. aureus* airway infection. *Am. J. Respir. Crit. Care Med.* **203**, 1127–1137 (2021).
136. Young, B. C. et al. Evolutionary dynamics of *Staphylococcus aureus* during progression from carriage to disease. *Proc. Natl Acad. Sci. USA* **109**, 4550–4555 (2012).
137. Young, B. C. et al. Severe infections emerge from commensal bacteria by adaptive evolution. *eLife* **6**, e30637 (2017).
138. Altman, D. R. et al. Genome plasticity of agr-defective *Staphylococcus aureus* during clinical infection. *Infect. Immun.* **86**, e00331-18 (2018).
139. Das, S. et al. Natural mutations in a *Staphylococcus aureus* virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation. *Proc. Natl Acad. Sci. USA* **113**, E3101–E3110 (2016).
140. Paudel, A., Panthee, S., Hamamoto, H., Grunert, T. & Sekimizu, K. YjbH regulates virulence genes expression and oxidative stress resistance in *Staphylococcus aureus*. *Virulence* **12**, 470–480 (2021).
141. Jousselin, A., Kelley, W. L., Barras, C., Lew, D. P. & Renzoni, A. The *Staphylococcus aureus* Thiol/oxidative stress global regulator Spx controls *trfA*, a gene implicated in cell wall antibiotic resistance. *Antimicrob. Agents Chemother.* **57**, 3283–3292 (2013).
142. Lopatkin, A. J. et al. Clinically relevant mutations in core metabolic genes confer antibiotic resistance. *Science* **371**, eaba0862 (2021).
143. Elgrail, M. M. et al. Convergent evolution of antibiotic tolerance in patients with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. *Infect. Immun.* **90**, e0000122 (2022).
144. Giulieri, S. G. et al. Comprehensive genomic investigation of adaptive mutations driving the low-level oxacillin resistance phenotype in *Staphylococcus aureus*. *mBio* **11**, e02882-20 (2020).
145. Howden, B. P., Johnson, P. D., Ward, P. B., Stinear, T. P. & Davies, J. K. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylococcus aureus bacteremia*. *Antimicrob. Agents Chemother.* **50**, 3039–3047 (2006).
146. Loss, G. et al. *Staphylococcus aureus* small colony variants (SCVs): news from a chronic prosthetic joint infection. *Front. Cell Infect. Microbiol.* **9**, 363 (2019).
147. Bär, J. et al. Quantification of within-patient *Staphylococcus aureus* phenotypic heterogeneity as a proxy for presence of persisters across clinical presentations. *Clin. Microbiol. Infect.* **28**, 1022.e1–1022.e7 (2022).
148. Laabei, M. et al. Evolutionary trade-offs underlie the multi-faceted virulence of *Staphylococcus aureus*. *PLoS Biol.* **13**, e1002229 (2015).
149. Xiong, Y. Q. et al. Phenotypic and genotypic characteristics of persistent methicillin-resistant *Staphylococcus aureus* bacteremia in vitro and in an experimental endocarditis model. *J. Infect. Dis.* **199**, 201–208 (2009).
150. Scott, W. K. et al. Human genetic variation in GLS2 is associated with development of complicated *Staphylococcus aureus* bacteremia. *PLoS Genet.* **14**, e1007667 (2018).
151. Spaan, A. N. et al. Human OTULIN haploinsufficiency impairs cell-intrinsic immunity to staphylococcal alpha-toxin. *Science* **376**, eabm6380 (2022).
This is the first evidence of a human heterozygous gene deficiency predisposing patients to severe *S. aureus* infection.
152. Chang, Y. L. et al. Human DNA methylation signatures differentiate persistent from resolving MRSA bacteremia. *Proc. Natl Acad. Sci. USA* **118**, e2000663118 (2021).
153. Ford, C. A., Hurford, I. M. & Cassat, J. E. Antivirulence strategies for the treatment of *Staphylococcus aureus* infections: a mini review. *Front. Microbiol.* **11**, 632706 (2020).
154. Francois, B. et al. Safety and tolerability of a single administration of AR-301, a human monoclonal antibody, in ICU patients with severe pneumonia caused by *Staphylococcus aureus*: first-in-human trial. *Intensive Care Med.* **44**, 1787–1796 (2018).
155. Magyarics, Z. et al. Randomized, double-blind, placebo-controlled, single-ascending-dose study of the penetration of a monoclonal antibody combination (ASN100) targeting *Staphylococcus aureus* cytotoxins in the lung epithelial lining fluid of healthy volunteers. *Antimicrob. Agents Chemother.* **63**, e00350-19 (2019).
156. Chan, R. et al. Identification of biologic agents to neutralize the bicomponent leukocidins of *Staphylococcus aureus*. *Sci. Transl Med.* **11**, eaat0882 (2019).
157. Mansson, M. et al. Inhibition of virulence gene expression in *Staphylococcus aureus* by novel depsipeptides from a marine photobacterium. *Mar. Drugs* **9**, 2537–2552 (2011).
158. Gao, P., Davies, J. & Kao, R. Y. T. Dehydrosqualene desaturase as a novel target for anti-virulence therapy against *Staphylococcus aureus*. *mBio* **8**, e01224-17 (2017).
159. Chen, X., Schneewind, O. & Missiakas, D. Engineered human antibodies for the opsonization and killing of *Staphylococcus aureus*. *Proc. Natl Acad. Sci. USA* **119**, e2114478119 (2022).
160. Miller, L. S., Fowler, V. G., Shukla, S. K., Rose, W. E. & Proctor, R. A. Development of a vaccine against *Staphylococcus aureus* invasive infections: evidence based on human immunity, genetics and bacterial evasion mechanisms. *FEMS Microbiol. Rev.* **44**, 123–153 (2020).

161. Tsai, C. M. et al. Non-protective immune imprint underlies failure of *Staphylococcus aureus* IsdB vaccine. *Cell Host Microbe* **30**, 1163–1172 (2022).
162. Nakatsuji, T. et al. Development of a human skin commensal microbe for bacteriotherapy of atopic dermatitis and use in a phase 1 randomized clinical trial. *Nat. Med.* **27**, 700–709 (2021).
Clinical study of bacteriotherapy highlighting that the inhibition of PSM production by commensal *S. hominis* decreases *S. aureus* colonization in atopic dermatitis patients.
163. Liu, Y. et al. Skin microbiota analysis-inspired development of novel anti-infectives. *Microbiome* **8**, 85 (2020).
164. Piewngam, P. & Otto, M. Probiotics to prevent *Staphylococcus aureus* disease? *Gut Microbes* **11**, 94–101 (2020).
165. Olagnier, D. et al. SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. *Nat. Commun.* **11**, 4938 (2020).
166. Horn, C. M. & Kielian, T. Crosstalk between *Staphylococcus aureus* and innate immunity: focus on immunometabolism. *Front. Immunol.* **11**, 621750 (2020).
167. Prince, A. & Wong Fok Lung, T. Consequences of metabolic interactions during *Staphylococcus aureus* infection. *Toxins* **12**, 581 (2020).
168. Thurlow, L. R. et al. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J. Immunol.* **186**, 6585–6596 (2011).
169. Pleasance, E. et al. Whole genome and transcriptome analysis enhances precision cancer treatment options. *Ann. Oncol.* **33**, 939–949 (2022).
170. Recker, M. et al. Clonal differences in *Staphylococcus aureus* bacteraemia-associated mortality. *Nat. Microbiol.* **2**, 1381–1388 (2017).
171. Young, B. C. et al. Antimicrobial resistance determinants are associated with *Staphylococcus aureus* bacteraemia and adaptation to the healthcare environment: a bacterial genome-wide association study. *Microb. Genom.* **7**, 000700 (2021).
172. Lilje, B. et al. Whole-genome sequencing of bloodstream *Staphylococcus aureus* isolates does not distinguish bacteraemia from endocarditis. *Microb. Genom.* https://doi.org/10.1099/mgen.0.000138 (2017).
173. Denamur, E. et al. Genome wide association study of *Escherichia coli* bloodstream infection isolates identifies genetic determinants for the portal of entry but not fatal outcome. *PLoS Genet.* **18**, e1010112 (2022).
174. Wozniak, J. M. et al. Mortality risk profiling of *Staphylococcus aureus* bacteremia by multi-omic serum analysis reveals early predictive and pathogenic signatures. *Cell* **182**, 1311–1327 (2020).

Acknowledgements
This work was supported by the National Health and Medical Research Council Australia through Investigator Grants to B.P.H. (GNT1196103) and T.P.S. (GNT1194325).

Author contributions
B.P.H., T.P.S., S.G.G., S.L.B., L.K.S., J.Y.H.L., A.H., I.R.M. and T.W.F.L. researched data for the article. B.P.H., T.P.S., S.G.G., S.L.B., J.Y.H.L., A.H., I.R.M. and T.W.F.L. substantially contributed to discussion of content. B.P.H. and T.P.S. led the writing of the article with contributions from S.G.G., J.Y.H.L., A.H., I.R.M. and T.W.F.L. B.P.H., T.P.S., S.G.G., L.K.S., A.H., I.R.M. and T.W.F.L. reviewed and edited the article.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41579-023-00852-y.

Correspondence should be addressed to Benjamin P. Howden.

Peer review information *Nature Reviews Microbiology* thanks Jos van Strijp, Timothy Foster and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.