Host-induced cell wall remodelling impairs opsonophagocytosis of *Staphylococcus aureus* by neutrophils

Elizabeth V. K. Ledger[1] and Andrew M. Edwards[1*]

[1] Centre for Bacterial Resistance Biology, Imperial College London, Armstrong Rd, London, SW7 2AZ, UK.

[*] For correspondence:
a.edwards@imperial.ac.uk

Keywords: *Staphylococcus aureus* / neutrophil / antibody / peptidoglycan / opsonophagocytosis / immune evasion

Running title: Cell wall accumulation conceals opsonins

Summary

The bacterial pathogen *Staphylococcus aureus* adapts to the host environment by increasing the thickness of its cell wall. However, the impact of cell wall thickening on susceptibility to host defences is unclear. Here, we show that as bacteria adapted to serum, the resulting increase in cell wall thickness led to a reduction in the exposure of bound antibody and complement and a corresponding reduction in phagocytosis and killing by neutrophils. The exposure of opsonins bound to protein antigens or LTA were most significantly reduced, whilst opsonisation by IgG against wall teichoic acid or peptidoglycan was largely unaffected. Partial digestion of accumulated cell wall in host adapted cells using the enzyme lysostaphin restored opsonin exposure and promoted phagocytosis and killing. Concordantly, the antibiotic fosfomycin inhibited cell wall remodelling and maintained full susceptibility of *S. aureus* to opsonophagocytic killing by neutrophils. These findings reveal that the adaptation of *S. aureus* to the host environment reduces the ability of the immune system to detect and kill this pathogen through reduced exposure of protein- and LTA-bound opsonins via cell wall remodelling.

Introduction

Staphylococcus aureus frequently infects wounds caused by surgery or insertion of intravenous access devices [1,2]. These infections can result in *S. aureus* seeding into the bloodstream, leading to bacteraemia and subsequent metastatic dissemination to sites including the heart, bones and joints [1,3,4,5]. Despite antibiotic therapy and a potent immune response, *S. aureus* infections have a high rate of relapse and frequently become chronic or recurrent [3,5].

Neutrophils are a key host defence against *S. aureus* infection and are recruited to the site of infection from the bloodstream [6,7,8,9,10,11]. The detection of *S. aureus* by neutrophils is largely dependent upon opsonisation of bacteria by bound antibody and complement, which is enabled in most people by the presence of antibodies against a range of different staphylococcal surface structures, including wall and lipoteichoic acids (WTA, LTA), peptidoglycan, capsular polysaccharide and proteins [10,11,12,13,14,15,16,17,18]. Whilst the precise abundance of antibody against each of the major surface structures varies from person to person, antibody against each macromolecule has been demonstrated to be sufficient to trigger opsonophagocytosis [14,15,16,17,18,19,20].

The binding of neutrophils to opsonins on the surface of *S. aureus* occurs via dedicated receptors and triggers phagocytosis of the pathogen followed by the subsequent exposure of ingested bacteria

62 to a raft of bactericidal products including reactive oxygen species, antimicrobial peptides and
63 proteases [11,12].

64 To combat the threat posed by neutrophils, *S. aureus* has evolved numerous mechanisms of evading
65 opsonic complement and antibody [12,13,21,22]. For example, *S. aureus* produces two
66 immunoglobulin binding proteins, Spa and Sbi, that reduce antibody-mediated opsonisation, whilst
67 the production of proteins such as SCIN, Efb and CHIPS reduces complement deposition and
68 activation and detection by immune cells [12,13,21,22,23,24,25,26,27,28,29,30,31]. As such, the
69 bacterial cell surface is a critically important determinant in immune detection of *S. aureus* and
70 efforts by the pathogen to evade surveillance and killing by host defences.

71 The staphylococcal cell envelope is a dynamic structure that responds to host-induced stresses
72 [32,33,34,35]. Consequently, *S. aureus* has a thicker cell wall *in vivo* than when growing *in vitro* [36],
73 a phenotype that is replicated when staphylococci are exposed to human serum or present within
74 endothelial or osteoblast cells [37,38,39,40]. In the case of serum, cell wall thickening is triggered
75 when *S. aureus* detects the presence of the host defence antimicrobial peptide LL-37 via the GraRS
76 two component system [37]. This results in significantly greater quantities of both peptidoglycan and
77 WTA in the cell wall, relative to bacteria grown in laboratory culture medium [37]. Importantly, the
78 changes to the cell envelope triggered by human serum are distinct from those that occur during
79 bacterial entry into stationary phase and are also not triggered by incubation of *S. aureus* in PBS or
80 cell culture medium. i.e. serum-induced changes are not simply due to a lack of nutrients or lack of
81 staphylococcal replication, but represent a specific response to the host environment [37].

82 Host-induced changes to the cell wall are important for the ability of the pathogen to cause and
83 sustain infection. Cell wall thickening has been shown to reduce susceptibility to antibiotics, whilst
84 mutant strains lacking various cell wall synthetic enzymes are less virulent in infection models
85 [32,34,35,37,40]. However, it is unknown whether host-induced changes to the bacterial cell wall
86 affect the detection and killing of *S. aureus* by the host immune system. To address this, we
87 examined the impact of host-induced changes to the staphylococcal cell envelope on subsequent
88 interactions of *S. aureus* with neutrophils. This revealed that cell wall thickening constitutes a
89 previously unrecognised mechanism of immune evasion that functions by significantly reducing the
90 exposure of opsonins bound to proteins and LTA and thereby reducing opsonophagocytic killing.

91

92

93

Results

Host adaptation reduces killing of *S. aureus* by neutrophils

To understand the impact of host adaptation on staphylococcal susceptibility to host defences, we either grew bacteria to exponential phase in tryptic soy broth (TSB) to represent standard laboratory conditions or allowed bacteria to adapt to host conditions using a previously described *ex vivo* human serum model that triggers cell wall thickening [37] (Fig. 1A). This model uses pooled human serum, which avoids variability in anti-staphylococcal antibody levels between donors [14]. Since the serum is not heat inactivated, it contains functional immunoglobulins and complement components.

In addition to triggering cell wall thickening via the GraRS system, serum also suppresses both the growth of *S. aureus* and activation of the Agr quorum-sensing system that regulates expression of many virulence factors [37,41,42,43,44,45,46,47,48].

We then measured the survival of bacteria prepared under each condition during incubation with purified *ex vivo* human neutrophils from male and female healthy donors in the presence of serum to provide antibody- and complement-mediated opsonisation [50,51]. We examined four distinct wild type *S. aureus* strains to represent both methicillin resistant (USA300, Col) and methicillin susceptible (SH1000, Newman) organisms [52,53,54,55].

For all four of the *S. aureus* strains tested, exponential phase bacteria were efficiently killed over time, with <5% of bacteria remaining viable after 2 hours incubation with neutrophils (Fig. 1B,C,D,E). However, host-adapted bacteria survived at levels up to 5 times greater than that seen for exponential bacteria for all strains (Fig. 1B,C,D,E). In addition to demonstrating that host adaptation reduces staphylococcal susceptibility to host immune defences, the high level of consistency observed across all four strains indicated that this is a conserved phenotype.

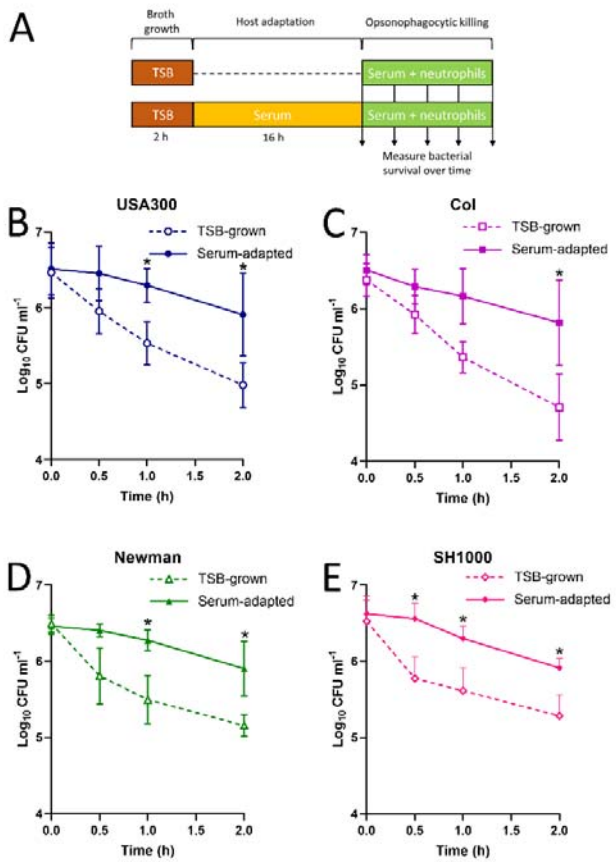


Figure 1. Incubation in human serum promotes tolerance to killing by neutrophils. Bacteria were grown in broth or allowed to adapt to human serum before incubation with human neutrophils in the presence of serum and staphylococcal survival measured over time (**A**). Survival of TSB-grown and serum-adapted cultures of *S. aureus* USA300 (**B**), Col (**C**), Newman (**D**) and SH1000 (**E**) during a 2 h incubation with purified human neutrophils. Data represent the geometric mean ± geometric standard deviation of at least three independent biological replicates. Data were analysed by a two-way ANOVA with Sidak's *post-hoc* test (*, $P < 0.05$; Serum-adapted vs TSB-grown at indicated time-points).

Host adaptation reduces opsonin exposure and opsonophagocytosis

Having found that host adaptation reduced staphylococcal susceptibility to host defences, we next determined the mechanism(s) responsible. Given the consistency in survival data across all four strains examined, we focussed on the USA300 lineage since it is both well characterised and clinically important [52].

We started by assessing whether the increase in survival of host-adapted bacteria was due to impaired phagocytosis, using two distinct assays. Bacteria were grown in broth or host-adapted by incubation in serum, before washing in PBS and then incubation with neutrophils in the presence of fresh serum to enable opsonisation (Fig. 2A). The first phagocytosis assay was a flow cytometry-

137 based approach that determined how many fluorescently-labelled bacteria were associated (or not)
138 with neutrophils [56] (Supplementary figure S1). This revealed that the majority of both broth-grown
139 and host-adapted *S. aureus* were phagocytosed after 30 min incubation with neutrophils. However,
140 whilst <3% of broth-grown bacteria remained unphagocytosed, >20% of host-adapted bacteria were
141 free from neutrophils (Fig. 2B). This finding was replicated in a second phagocytosis assay that
142 measured the viability of free and neutrophil-associated bacteria [57], with >10% of host-adapted *S.*
143 *aureus* unphagocytosed compared with <1% of broth-grown *S. aureus* cells (Fig. 2C; Supplementary
144 figure S2). Combined, these two assays demonstrated that host-adapted *S. aureus* were significantly
145 better at evading phagocytosis than broth-grown bacteria.

146 An additional finding was that there were equal numbers of viable host-adapted and broth-grown *S.*
147 *aureus* cells associated with neutrophils. This indicated that host-adapted *S. aureus* were as
148 susceptible to the microbicides produced by the neutrophils as broth-grown cells (Fig. 2C).
149 Therefore, we concluded that the enhanced survival of host-adapted bacteria compared with broth-
150 grown bacteria (Fig. 1) was due to enhanced evasion of phagocytosis, rather than resistance to the
151 antibacterial products of neutrophils.

152 To understand why more host-adapted *S. aureus* were able to evade phagocytosis compared with
153 broth-grown cells, we examined the degree of opsonisation of bacteria by antibody and complement
154 using western blotting. In keeping with previous work [58,59], for this experiment we used a mutant
155 strain of USA300 lacking Spa and Sbi to avoid interference caused by these immunoglobulin binding
156 proteins (Supplementary Fig. S3). Broth-grown or host-adapted bacteria were washed in PBS and
157 then incubated, or not, in fresh serum to enable opsonin binding as used in the opsonophagocytosis
158 assays described above (Fig. 1A) before detection of bound antibody and complement component
159 C3 (Fig. 2D).

160 Despite their reduced phagocytosis by neutrophils, there was more antibody and complement
161 bound to host-adapted cells than to broth-grown, suggesting that a lack of bound opsonins did not
162 explain the immune evasion phenotype of host-adapted bacteria (Supplementary Fig. S4).

163 To understand why host-adapted cells had high levels of bound antibody and complement but low
164 levels of phagocytosis, bacteria were prepared as described above for opsonophagocytosis assays
165 and then the levels of surface-exposed antibody and the complement component C3 quantified
166 using flow cytometry (Fig. 2D; Supplementary Fig. S5). Broth-grown bacteria that had been exposed
167 to PBS instead of serum acted as a negative control and confirmed that antibodies used in the assay
168 did not bind non-specifically to *S. aureus* cells (Fig. 2D,E,F). We then showed that, as expected,

169 broth-grown bacteria that were incubated in human serum for 30 min were very strongly bound by
170 both IgG and the complement component C3 (Fig. 2E,F).

171 Next, we examined host-adapted bacteria and found that they had a significantly reduced level of
172 exposed opsonins, compared with TSB-grown cells, regardless of whether they had been incubated
173 in fresh serum for 30 min or not (Fig. 2E,F). Therefore, despite prolonged incubation in serum and
174 high levels of bound antibody and complement (Supplementary Fig. S4), host-adapted cells had
175 significantly reduced exposure of opsonins on their cell surface relative to broth-grown bacteria that
176 had been opsonised.

177 Taken together, these experiments revealed that host-adapted bacteria are better able to survive
178 exposure to neutrophils than broth-grown *S. aureus* because they are less likely to be phagocytosed,
179 in keeping with the lower surface exposure of bound IgG and complement.

180

181

182

183

184

185

186

187

188

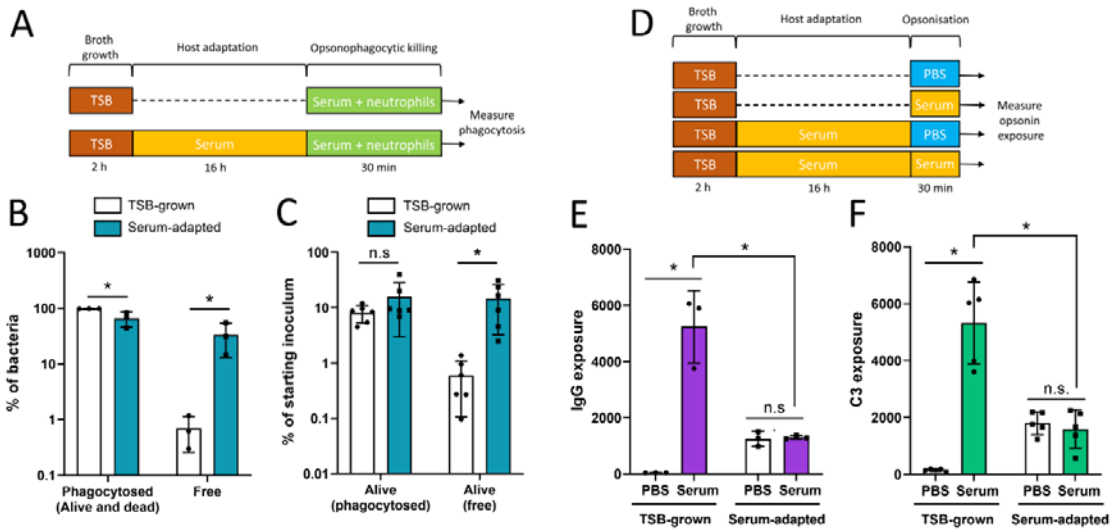


Figure 2. Incubation in human serum reduces opsonophagocytosis and surface-exposed IgG and complement. TSB-grown and serum-adapted cultures of *S. aureus* USA300 were incubated with purified human neutrophils for 30 min (**A**) before the percentage of neutrophil-associated (phagocytosed) and non-neutrophil associated (free) bacteria were determined by (**B**) flow cytometry and (**C**) CFU counts. TSB-grown and serum-adapted *S. aureus* cells were incubated for 30 min with PBS or 10 % serum (**D**) before the levels of surface-exposed bound (**E**) IgG and (**F**) C3 were determined by flow cytometry. Data in **B** and **C** show values for each independent experiment, with bars representing the mean ± standard deviation. In **E** and **F**, each biological repeat is represented by the median fluorescence value of 10,000 bacterial cells. Data in **B, C, E, F** were analysed by two-way ANOVA with Sidak's *post-hoc* test (*, $P < 0.05$; n.s, $P \geq 0.05$ for the indicated comparisons).

Cell wall accumulation impairs opsonophagocytosis by concealing IgG bound to LTA and protein

Since the cell envelope of *S. aureus* accumulates peptidoglycan and WTA during incubation in serum [37], we tested whether this concealed some of the bound antibody and complement. To do this, host-adapted bacteria were incubated for 20 min with a range of sub-lethal concentrations of the enzyme lysostaphin, which cleaves peptidoglycan, to partially remove the cell wall. The lysostaphin was then removed by washing and bacterial viability confirmed by CFU counts. This limited cell wall digestion resulted in a significant, dose-dependent increase in exposure of bound IgG and complement, demonstrating that some of the bound opsonins were concealed by the accumulation of cell wall polymers during incubation in serum (Fig. 3A,B).

We then tested whether the concealment of bound IgG by accumulated cell wall explained the reduced phagocytosis of host adapted bacteria relative to TSB-grown *S. aureus*. In keeping with increased IgG and complement exposure, limited lysostaphin treatment increased the phagocytosis of host-adapted *S. aureus* by neutrophils (Fig. 3C,D).

215 Since human serum contains IgG that recognises multiple *S. aureus* surface structures, we next
216 sought to understand whether the reduced opsonisation observed for serum-adapted bacteria was
217 specific to a particular antibody target. Bacteria were grown in TSB and then incubated briefly in
218 serum (30 min) or for 16 h to enable adaptation. Surface exposed IgG was then eluted from bacteria
219 and assessed for its binding to each of the major surface structures by ELISA.

220 Host-adapted bacteria had similar levels of anti-WTA IgG on their surface compared to exponential
221 phase bacteria and only slightly lower levels of anti-peptidoglycan IgG (2.5-fold difference).
222 However, surface exposure of IgG targeting other surface structures was greatly reduced in host-
223 adapted bacteria compared to exponential phase cells, with anti-LTA IgG 9-fold lower, anti-
224 membrane-associated proteins 18-fold lower and cell wall-associated proteins 48-fold lower (Fig.
225 3E). We did not examine capsular polysaccharide in these assays as USA300 is deficient in this
226 polymer [52]. As such, the lower surface IgG exposure in host-adapted cells compared to those in
227 exponential phase is primarily due to a loss of exposure of antibody bound to LTA and surface
228 proteins.

229 Partial digestion of peptidoglycan using lysostaphin restored surface exposure of IgG bound to LTA
230 and proteins to similar levels observed for TSB-grown bacteria (Fig. 3E). Therefore, accumulation of
231 cell wall in host-adapted bacteria preferentially conceals IgG bound to LTA and surface proteins,
232 whilst exposure of anti-WTA and anti-peptidoglycan remains strongly exposed.

233 Finally, we showed that increasing opsonin exposure via partial lysostaphin digestion of the cell wall,
234 with the enzyme washed away before incubation with immune cells, rendered host adapted
235 staphylococci as susceptible to neutrophil-mediated killing as broth-grown bacteria (Fig. 3F).

236 Taken together, the experiments described here demonstrate that host-adapted *S. aureus* cells are
237 bound by high levels of antibody and complement but the accumulation of cell wall conceals some
238 of these bound opsonins, reducing phagocytosis and killing by neutrophils.

239

240

241

242

243

244

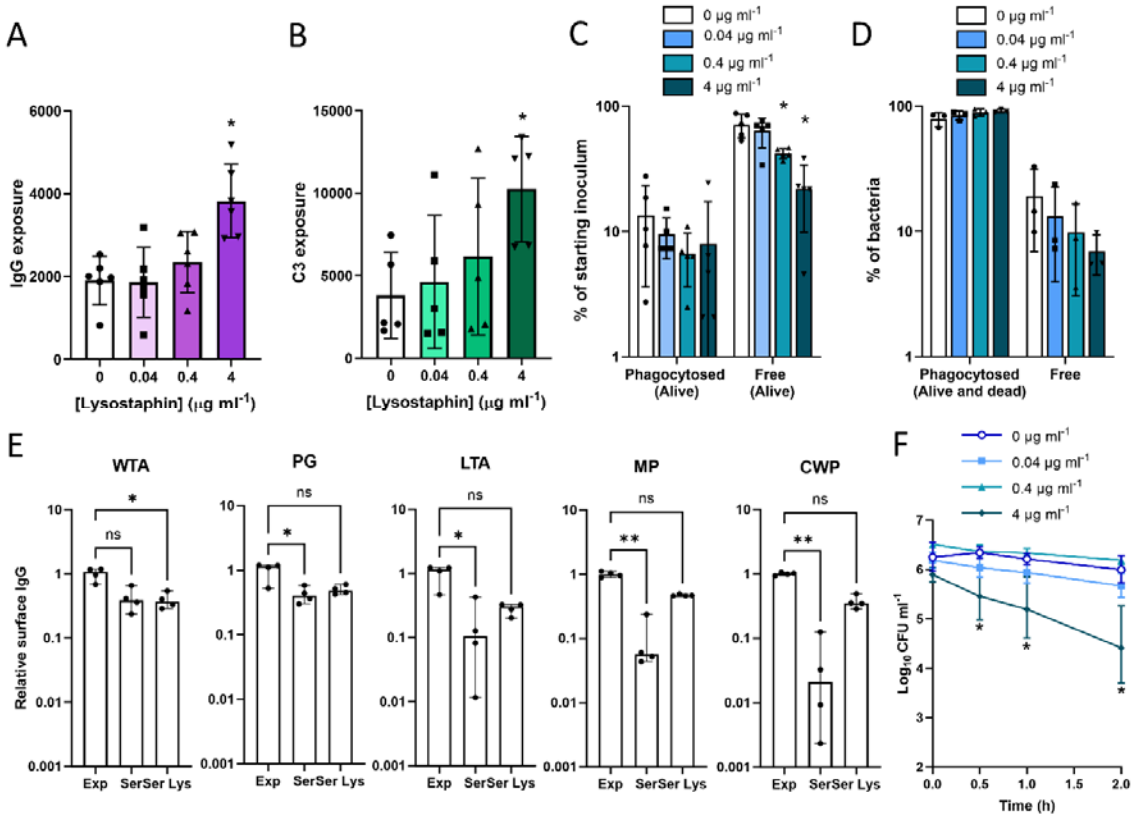


Figure 3. Cell wall accumulation impairs opsonophagocytosis by concealing bound opsonins. Host-adapted cultures of *S. aureus* USA300 were treated with indicated concentrations of lysostaphin for 20 min to partially digest the cell wall before levels of surface-exposed (**A**) IgG and (**B**) complement component C3 were determined by flow cytometry. Additionally, these cells were incubated with purified human neutrophils for 30 min before the percentage of neutrophil-associated (phagocytosed) and non-neutrophil associated (free) bacteria were determined by (**C**) CFU counts and (**D**) flow cytometry. (**E**) Binding ligands of IgG eluted from the surface of *S. aureus* grown to exponential phase and then incubated in serum for 30 min (Exp), allowed to adapt to serum (Ser) or serum-adapted cells that were subsequently subjected to lysostaphin treatment as determined by ELISA. Antibodies were assessed for binding to wall teichoic acid (WTA), peptidoglycan (PG), lipoteichoic acid (LTA), membrane-associated proteins (MP) or cell wall-associated proteins (CWP). (**F**) Survival of lysostaphin-treated cells during a 3 h incubation with purified human neutrophils. Lysostaphin was washed away before incubation with neutrophils.

Data in **A** – **D** represent the mean ± standard deviation of the indicated number of independent biological replicates. In **A** and **B**, each biological repeat is represented by the median fluorescence value of 10,000 bacterial events. Data in **A** and **B** were analysed by one-way ANOVA with Dunnett's *post-hoc* test. Data in **C**, **D** and **F** were analysed by two-way ANOVA with Dunnett's *post-hoc* test (*, $P \leq 0.05$; Lysostaphin-treated vs non-lysostaphin treated). Data in **E** represent the median ± 95% CI of four independent biological replicates and were analysed by Kruskal Wallis test and Dunn's *post-hoc* test to establish statistically significant differences between groups (**, $P < 0.01$; *, $P < 0.05$; ns, $P \geq 0.05$ for the indicated comparisons). Data in **F** represent the geometric mean ± geometric standard deviation of 4 independent experiments.

271
272
273
274 **Antibiotic-mediated inhibition of peptidoglycan accumulation maintains opsonin exposure and**
275 **efficient opsonophagocytosis**

276 To further test whether host adaptation reduced phagocytosis via cell wall-mediated concealment of
277 bound opsonins, and to explore potential therapeutic approaches to enhance neutrophil-mediated
278 killing, we first used the antibiotic fosfomycin to block the accumulation of peptidolgycan during
279 host-adaptation, as we have done previously [37]. This antibiotic targets MurA, which catalyses the
280 production of the peptidoglycan precursor UDP *N*-acetylmuramic acid in the cytoplasm [60]. This
281 inhibits peptidoglycan synthesis and prevents serum-induced cell wall thickening from occurring and
282 has been used clinically in anti-staphylococcal combination therapies [37,61,62].

283 As observed previously, the adaptation of bacteria to serum resulted in a significant reduction in
284 opsonisation, as determined by exposure of IgG and complement, relative to broth-grown bacteria
285 (Fig. 4A,B). However, the presence of fosfomycin in serum significantly reduced opsonin
286 concealment, maintaining IgG and complement exposure at similar levels to that seen for broth-
287 grown bacteria (Fig. 4A,B).

288 Further analysis of IgG exposure confirmed that fosfomycin treatment preserved the exposure of IgG
289 bound to all major surface structures relative to bacteria that had not been treated with the
290 antibiotic (Supplementary Fig. S6). Similar findings occurred with another inhibitor of peptidoglycan
291 synthesis, oxacillin, which acts on penicillin binding proteins [63], whereas antibiotics that targeted
292 fatty acid biosynthesis (AFN-1252) or DNA gyrase (ciprofloxacin) did not increase IgG exposure
293 relative to host adapted cells that had not been exposed to antibiotics [64,65] (Supplementary Fig.
294 S6). Therefore, in support of our previous findings, we concluded that the accumulation of
295 peptidoglycan during host adaptation significantly reduces the exposure of IgG bound to LTA and
296 surface proteins.

297 The increased exposure of IgG and complement on the surface of bacteria incubated in serum
298 containing fosfomycin, restored phagocytosis to levels seen with broth-grown bacteria, as
299 determined by both phagocytosis assays (Fig. 4C,D). Furthermore, bacteria that were adapted in
300 serum with fosfomycin were killed by neutrophils as efficiently as broth-grown bacteria, whereas
301 host-adapted bacteria not exposed to fosfomycin survived at significantly higher levels (Fig. 4E). In
302 keeping with our analysis that AFN-1252 did not prevent IgG concealment during host adaptation,
303 this antibiotic did not promote phagocytosis of host-adapted *S. aureus* (Supplementary Fig. S7).

304 *S. aureus* anchors proteins to peptidoglycan via sortase enzymes (SrtA and SrtB) and so we assessed
305 whether the impact of fosfomycin on host adaption was due to interference with this process.
306 However, host-adapted mutants defective for SrtA or SrtB, which cannot anchor proteins to
307 peptidoglycan, survived incubation with neutrophils as well as host-adapted wild type bacteria
308 (Supplementary Fig. S8A,B).

309 Taken together, these findings provided additional evidence that cell wall accumulation conceals
310 opsonins bound to LTA and surface proteins, which in turn compromises phagocytosis and killing by
311 neutrophils. They also indicate that the antibiotic fosfomycin, in addition to its own antibacterial
312 activity, may aid the clearance of infection by preventing concealment of opsonins.

313

314

315

316

317

318

319

320

321

322

323

324

325

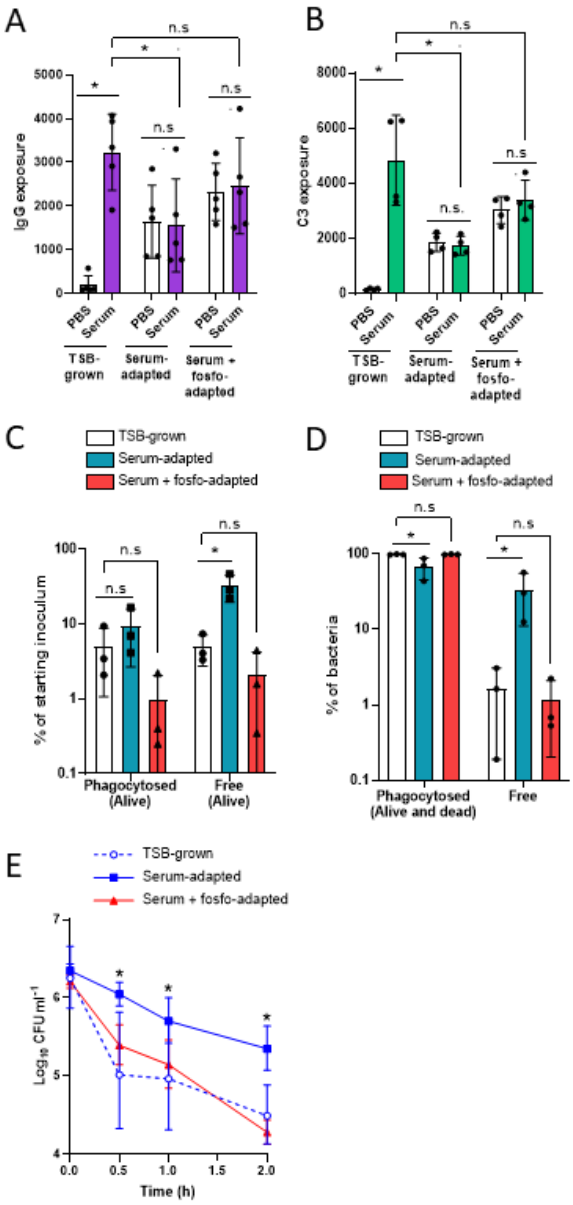
326

327

328

329

330



331

332 **Figure 4. Antibiotic-mediated inhibition of cell wall accumulation maintains opsonin exposure and**
333 **efficient opsonophagocytosis.** TSB-grown, serum-adapted and serum + fosfomycin-adapted cultures
334 of *S. aureus* USA300 were incubated for 30 min with PBS or 10 % serum before the levels of surface-
335 exposed (**A**) IgG and (**B**) complement component C3 were determined by flow cytometry.
336 Additionally, these cells were incubated with purified human neutrophils for 30 min before the
337 percentage of neutrophil-associated (phagocytosed) and non-neutrophil associated (free) bacteria
338 were determined by (**C**) CFU counts and (**D**) flow cytometry. (**E**) Survival of TSB-grown, serum-
339 adapted and serum + fosfomycin-adapted cultures during a 2 h incubation with purified human
340 neutrophils. Summary diagram of the effect of fosfomycin on opsonin exposure during host-

341 adaptation, with antibiotic-mediated inhibition of peptidoglycan accumulation preserving the
342 exposure of bound antibody and complement (**F**). Data in **A** – **D** represent the geometric mean ±
343 geometric standard deviation of the indicated number of independent biological replicates. Data in **E**
344 are presented as the geometric mean ± geometric standard deviation of three independent
345 experiments. In **A** and **B**, each biological repeat is represented by the median fluorescence value of
346 10,000 bacterial events. Data in **A** and **B** were analysed by two-way ANOVA with Tukey's *post-hoc*
347 test. Data in **C** – **E** were analysed by two-way ANOVA with Dunnett's *post-hoc* test (*, $P < 0.05$; n.s, P
348 ≥ 0.05; comparisons are indicated in **A** – **D** and serum/serum + fosfomycin-adapted vs TSB-grown at
349 each time-point in **E**).

350

351 **Discussion**

352 The binding of antibody and complement to the bacterial cell surface enables the detection and
353 destruction of pathogens by phagocytic immune cells [10,11,13]. The data presented here
354 demonstrate that *S. aureus* can conceal a subset of bound opsonins via cell wall accumulation,
355 significantly reducing opsonophagocytosis and killing by neutrophils, a previously unrecognised
356 mechanism of immune evasion (Supplementary Fig. S9).

357 Cell wall remodelling occurs in response to host stresses and provides protection against antibiotics
358 and host defence peptides. It involves the accumulation of peptidoglycan and WTA [36,37,66] and it
359 is therefore unsurprising that exposure of antibody targeting these two polymers was least affected
360 by host adaptation. By contrast, the exposure of antibodies bound to surface proteins and LTA was
361 significantly reduced by host adaptation, in keeping with their localisation within the cell wall itself
362 [67].

363 Previous work indicated that WTA can block antibodies from binding to antigens within the cell wall
364 [68]. Although WTA accumulates in the wall during host adaptation, it is currently unknown whether
365 this contributes to concealment of IgG bound to LTA or proteins. Unfortunately, since cell wall
366 accumulation is dependent upon D-alanine labelled WTA [37], we could not use a WTA-deficient
367 mutant to explore the role of this polymer in reducing opsonin exposure. However, our work did
368 show that inhibition of peptidoglycan accumulation preserved antibody exposure and
369 opsonophagocytic killing by neutrophils, demonstrating a key role for cell wall accumulation.

370 During host adaptation, peptidoglycan accumulation is due to a combination of peptidoglycan
371 synthesis and inhibition of autolytic activity [37]. Recent work has revealed that mutants lacking the
372 Atl autolysin have defective surface exposure of staphylococcal surface proteins, inhibiting their

373 recognition by reactive antibodies [69]. Exposure of surface proteins was restored using enzymatic
374 digestion of peptidoglycan, providing additional evidence that peptidoglycan accumulation can
375 obscure surface antigens and prevent their detection by antibodies. However, the impact of
376 concealment of surface proteins on opsonophagocytosis has not been investigated previously.

377 Several experimental vaccines have been developed based on surface proteins in an attempt to
378 generate high serum titres of opsonising antibody. Unfortunately, despite very promising data from
379 animal infection experiments, none of these vaccines have shown efficacy in humans [70]. Several
380 plausible reasons for this discrepancy have been proposed, including the host-specificity of
381 staphylococcal immune evasion factors and previous staphylococcal infection directing the host
382 towards non-protective immunity [14,70,71].

383 Another difference between model infection of animals and natural infection in humans is the
384 physiological state of the bacteria. For many animal infections, bacteria are grown in TSB
385 immediately prior to administration into the animal and will therefore have high levels of multiple
386 protein antigens exposed on their surface, which facilitates rapid opsonophagocytosis
387 [72,73,74,74,75]. By contrast, natural invasive infection typically begins with colonisation of
388 superficial sites such as an inserted IV catheter and so bacteria may be host-adapted when they
389 enter the bloodstream and are thus less well recognised by antibodies targeting surface proteins
390 [76,77,78,79,80]. As such, the addition of WTA as a vaccine antigen may provide a reasonable level
391 of protection against bacteria that have accumulated cell wall and thus have reduced exposure of
392 surface proteins.

393 Previous work has indicated that the thickened cell wall associated with vancomycin resistance
394 reduces staphylococcal susceptibility to intracellular killing by neutrophils [81]. However, our data
395 did not show a difference in staphylococcal survival within neutrophils, with similar numbers of
396 intracellular viable broth-grown and host-adapted bacteria. Instead, the survival advantage of host
397 adaptation appeared to be due to enhanced evasion of phagocytosis.

398 We do not yet know if these findings are applicable to other Gram-positive pathogens. However,
399 since previous work has shown that serum triggers cell wall thickening in *Enterococcus faecalis* and
400 viridans group streptococci, it is possible that our findings with *S. aureus* represent a broadly
401 conserved mechanism of immune evasion [82].

402 Cell wall thickening in *S. aureus* is triggered by bacterial sensing of the host defence antimicrobial
403 peptide LL-37 via the GraRS system [37]. Since LL-37 is present in most tissues and amongst the
404 earliest host responses to infection or trauma [81,82,83], we hypothesise that *S. aureus* has evolved

405 to sense this AMP as an early indicator that it is subject to immune attack and provides an
406 opportunity to employ defensive measures against the impending arrival of neutrophils. In support
407 of this hypothesis, GraRS, the two-component system that detects LL-37, is activated in the early
408 stages of staphylococcal skin colonisation, whilst *S. aureus* mutants lacking GraRS are significantly
409 less virulent than wild type strains in invasive infection models [84,85,86].

410 In addition to providing protection against opsonophagocytosis, LL-37 exposure triggers reduced
411 susceptibility to the antibiotics daptomycin and vancomycin [37,87], suggesting *S. aureus* employs
412 strategies that are broadly protective against the twin threats of host immunity and antibiotic
413 therapy. This is similar to our previous work showing that induction of the *S. aureus* general stress
414 response regulated by the alternative sigma factor SigB can promote survival of bacteria exposed to
415 host defences and various classes of antibiotics [88]. Further support for the link between *S. aureus*-
416 immune interactions and antibiotic tolerance comes from studies showing that oxidative stress
417 conferred by phagocytic cells reduce staphylococcal susceptibility to antibiotics [89,90].

418 Whilst the immune response may compromise the efficacy of antibiotic therapy under certain
419 circumstances, our study also highlights how antibiotics and the immune response can work
420 synergistically by showing that fosfomycin blocked LL-37 induced cell wall thickening and thereby
421 maintained exposure of bound opsonins, leading to efficient opsonophagocytic killing. Additionally,
422 previous work has suggested that fosfomycin also promotes killing of *S. aureus* via enhanced
423 production of the neutrophil oxidative burst [91]. However, whilst we exposed *S. aureus* to
424 fosfomycin in serum, this was removed by washing prior to incubation with neutrophils and thus
425 does not explain the enhanced killing effect observed in our assays. This strongly suggests that there
426 are at least two mechanisms by which fosfomycin and neutrophils synergise against *S. aureus* and a
427 greater understanding of this may contribute to more effective therapeutic approaches that reduce
428 the high incidence of relapsing or chronic staphylococcal infections [92].

429 In summary, we show that *S. aureus* cells are heavily opsonised upon initial exposure to serum.
430 However, *S. aureus* responds to serum by accumulating peptidoglycan, which conceals bound
431 opsonins, reducing phagocytosis and killing by neutrophils.

432

433

434

435

436

437

438 ## Materials and methods

439 ### Bacterial strains and growth conditions

440 Bacterial strains used in this study are shown in Table 1. Strains were grown at 37 °C on tryptic soy
441 agar (TSA) or in tryptic soy broth (TSB) with shaking (180 r.p.m.) supplemented with erythromycin
442 (10 µg ml^{-1}) or kanamycin (90 µg ml^{-1}) when required.

Strain	Relevant characteristics	Reference/source
USA300 JE2	LAC strain of the USA300 lineage of community acquired MRSA cured of plasmids	36
JE2 *sbi*::Tn *spa*::*kan*	JE2 strain defective for immunoglobulin-binding proteins Spa and Sbi	This study
JE2 *srtA*::Tn	JE2 strain defective for sortase A, which anchors LPxTG motif-containing proteins to peptidoglycan	93
JE2 *srtB*::Tn	JE2 strain defective for sortase B, which anchors NPQTN motif-containing IsdC protein to peptidoglycan	93
SH1000	*rsbU*+ derivative of laboratory strain 8325-4	37
Col	Early hospital-associated MRSA isolate, commonly used in laboratory experiments	38
Newman	Clinical isolate, methicillin susceptible, used widely in animal and laboratory experiments	39

443

444 **Table 1.** Strains used in this study.

445

446 ### Construction of strains

447 The JE2 *sbi*::Tn/*spa*::*kan* double mutant was constructed via transduction of the kanamycin
448 resistance marker from Newman *spa*::*kan* [94] into the *sbi*::Tn mutant present in the NARSA
449 transposon mutant library [93] using ϕ11.

450

451 **IgG Fc binding assay**

452 The Fc portion of human Immunoglobulin G (1 mg, Abcam) was labelled with biotin (Thermo
453 Scientific EZ-Link Sulfo-NHS-Biotin) before removal of unbound biotin by dialysis. Labelled Fc portion
454 was then incubated with PBS washed bacterial cells for 30 min (10 µg protein and 10^9 CFU *S. aureus*
455 in 1 ml PBS). Unbound immunoglobulin fragment was removed by three rounds of washing with PBS
456 before cells were incubated with streptavidin-alkaline phosphatase for 30 min. Cells were then
457 washed with three rounds of PBS before incubation in 200 µl p-Nitrophenol phosphate substrate
458 solution for ELISA (Merck) for 10 min. Cells were then pelleted by centrifugation and the supernatant
459 recovered and A_{405} determined.

460 **Generation of broth-grown and host-adapted bacterial cultures**

461 To generate broth-grown bacteria, cultures were grown for 16 h in TSB to stationary phase. These
462 were then diluted to 10^7 CFU ml^{-1} in fresh TSB and incubated for 2 h at 37 °C until 10^8 CFU ml^{-1} was
463 reached.

464 To generate host-adapted bacteria, broth-grown cultures were centrifuged (3,200 x *g* for 10 min),
465 resuspended in an equal volume of human serum from human male AB plasma (Sigma) and
466 incubated for 16 h at 37 °C. As *S. aureus* is unable to replicate in human serum, these cultures were
467 also at 10^8 CFU ml^{-1} [37,47,48,49]. Where appropriate, serum was supplemented with a sub-lethal
468 concentration of fosfomycin (64 µg ml^{-1}), oxacillin (128 µg ml^{-1}), ciprofloxacin (160 µg ml^{-1}) or AFN-
469 1252 (0.15 µg ml^{-1}). These concentrations were chosen based on previous work that showed they
470 were the maximum concentration that did not affect staphylococcal viability in serum [37].
471

472 Where appropriate, the cell walls of serum-adapted cultures were degraded by lysostaphin. To do
473 this, 1 ml aliquots of serum-adapted bacteria were washed in PBS and resuspended in 1 ml PBS
474 supplemented with indicated concentrations of lysostaphin (between 0.04 and 4 µg ml^{-1}). Bacteria
475 were incubated statically for 20 min at 37 °C before being washed by 3 rounds of centrifugation in
476 PBS.

477 **Purification of neutrophils**

478 Ethical approval for drawing and using human blood was obtained from the Regional Ethics
479 Committee and the Imperial NHS Trust Tissue Bank (REC Wales approval no. 12/WA/0196 and ICHTB
480 HTA license no. 12275). Donors provided informed consent before donating. Neutrophils were
481 extracted from 45 ml human blood, collected in heparin tubes to prevent coagulation. Blood (15 ml)
482 was carefully layered over 30 °C PolymorphPrep (20 ml) and centrifuged for 1 h at 500 x *g* to

483 separate the different cell types. Neutrophils were collected, washed with Hanks balanced salt
484 solution (HBSS) and adjusted to 5 x 10^6 cells ml^{-1} in HBSS.

485 **Determination of bacterial killing by neutrophils and phagocytosis by CFU counts**

486 Neutrophils were adjusted to 5 x 10^6 cells ml^{-1} in HBSS supplemented with 10 % human serum, 0.1
487 mM $CaCl_2$ and 0.1 mM $MgCl_2$. In the case of lysostaphin-treated bacteria, 10 % serum was omitted
488 from the HBSS.

489 TSB-grown/host-adapted bacteria were generated as described above, washed three times in PBS
490 and then added to neutrophils at 5 x 10^6 CFU ml^{-1}. Tubes were incubated with end-over-end mixing
491 at 37 □ C for 3 h and at each time-point (0, 0.5, 1 and 2 h) aliquots were removed, serially diluted
492 10-fold in PBS and plated to enumerate CFU ml^{-1}.

493 In addition, the number of phagocytosed/unphagocytosed bacteria was also enumerated at the 0.5
494 h time-point. A 500 µl aliquot of the neutrophil/bacteria mixture was taken and centrifuged at 500 x
495 *g* for 1 min to pellet the neutrophils, along with any neutrophil-associated bacteria. The supernatant
496 (containing unphagocytosed bacteria) was serially diluted 10-fold in PBS and plated for CFU counts
497 and the pellet was resuspended in 500 µl PBS, serially diluted 10-fold in PBS and plated for CFU
498 counts. The CFU ml^{-1} values in the pellet and the supernatant were divided by the CFU ml^{-1} of the
499 starting inoculum to generate the percentage of CFU ml^{-1} phagocytosed and unphagocytosed,
500 respectively.

501 To validate the experimental conditions used in this assay, two control experiments were run. Firstly,
502 bacteria were prepared as described above were subjected to centrifugation and the CFU counts
503 pre- and post-centrifugation quantified to determine whether bacteria were pulled out of
504 suspension under these conditions. Secondly, to understand whether bacteria associated with
505 neutrophils were intracellular, bacteria were incubated with neutrophils as described above, before
506 subsequent incubation with or without lysostaphin (40 µg ml^{-1}) to kill extracellular bacteria.
507 Neutrophils were then washed and CFU counts determined before washing to remove the lytic
508 enzyme. In some experiments neutrophils were lysed with Triton X-100 (0.1%) to determine whether
509 this affected recovery of CFU.

510

511 **Measurement of phagocytosis by flow cytometry**

512 To measure phagocytosis by flow cytometry neutrophils and bacteria were prepared as described
513 above except that immediately before bacteria were added to the neutrophils the bacteria were

514 incubated with 10 µg ml^{-1} fluorescein isothiocyanate for 30 min at room temperature and then
515 washed three times in PBS.

516 As above, 5 x 10^6 CFU ml^{-1} bacteria were added to 5 x 10^6 cells ml^{-1} in HBSS supplemented with 10 %
517 human serum, 0.1 mM $CaCl_2$ and 0.1 mM $MgCl_2$. In the case of lysostaphin-treated bacteria, 10 %
518 serum was omitted from the HBSS. After a 30 min incubation at 37 □ C with end-over-end mixing in
519 the dark, cultures were fixed by the addition of an equal volume of 4 % paraformaldehyde (PFA).
520 Samples were then analysed by flow cytometry using an Amnis CellStream. Bacteria were detected
521 using the 488 nm laser and at least 10,000 bacterial events were recorded. Events with FITC ≥ 2 x 10^3
522 were counted as bacteria. Events with an FCS of ≥ 3000 were counted as phagocytosed and < 3000
523 were counted as unphagocytosed/free.

524

525 **Measurement of IgG and complement surface exposure by flow cytometry**

526 TSB-grown and serum-adapted cultures were prepared as described above. A *spa/sbi* double mutant
527 was used to prevent non-specific antibody binding. Aliquots (500 µl) were incubated for 30 min at
528 room temperature in either PBS or 10 % human serum. Samples were washed by three rounds of
529 centrifugation in PBS (13,000 x *g* for 1 min) and blocked for 1 h in 4 % BSA in PBS. Samples were
530 washed once in PBS before IgG was detected with a 1:1000 dilution of goat anti-human IgG antibody
531 labelled with the BV421 fluorophore (Jackson ImmunoResearch) or C3 was detected with a 1:1000
532 dilution of goat anti-human C3 $F(ab')_2$ labelled with FITC (Protos Immunoresearch). Antibody
533 incubations were carried out statically for 1 h at room temperature in the dark. Samples were
534 washed with PBS by three rounds of centrifugation (13,000 x *g* for 1 min) and fixed in 4 % PFA.
535 Samples were analysed by flow cytometry using an Amnis CellStream. IgG was detected using the
536 405 nm laser and C3 using the 488 nm laser. At least 10,000 bacterial events were recorded and the
537 median value recorded.

538 **Measurement of IgG and complement by western blotting**

539 Cultures of TSB-grown and serum-adapted bacteria (1 ml at 10^8 CFU ml^{-1}) were prepared as
540 described above, washed by three rounds of centrifugation in PBS (13,000 x *g* for 1 min) and
541 resuspended in 100 µl PBS. A *spa/sbi* double mutant was used to prevent non-specific antibody
542 binding. Lysostaphin (10 µg ml^{-1}) was added and bacteria incubated statically for 1 h at 37 □ C.
543 Sample buffer (187.5 mM Tris-HCl (pH 6.8), 6 % SDS, 30 % glycerol, 0.03 % bromophenol blue and 15
544 % beta-mercaptoethanol; 50 µl) was added and samples were incubated at 95 □ C for 10 min before
545 15 µl was loaded onto 10 % polyacrylamide gels. Gels were run in Tris-Glycine running buffer (25

546 mM Tris, 192 mM glycine, 0.1 % SDS, pH 8.4) at 100 V for 10 min followed by 200 V for 50 min before
547 being transferred onto PVDF membranes (10 V for 60 min). Membranes were blocked for 1 h at
548 room temperature in 5 % milk and 1 % BSA in TBST. IgG was detected using 1:10000 dilution of
549 donkey anti-human IgG conjugated to HRP (Abcam) and C3 was detected by 1:5000 dilution of rabbit
550 anti-C3 (Abcam) followed by 1:10000 dilution of goat anti-rabbit IgG conjugated to HRP (Abcam).
551 Blots were developed using SuperSignal West Pico PLUS chemiluminescent substrate (Thermo
552 scientific) and imaged using the Bio-rad ChemiDoc MP imaging system.
553
554 **Characterisation of IgG bound to cells**
555 Bacteria were grown to exponential phase and incubated in serum for 30 min or allowed to adapt
556 for 16 h, followed or not by partial cell wall digestion using lysostaphin as described above. Cells
557 (10^8) were washed three times in PBS before bound antibody was eluted using 200 µl antibody
558 elution buffer (Pierce) for 5 min. Cells were then removed by centrifugation and the eluted antibody
559 solution neutralised with 100 µl protein A binding buffer (Pierce).

560 To determine the binding ligands of bound antibodies, 10 µg purified cell surface components LTA
561 (Sigma), WTA [37], peptidoglycan [37], membrane proteins [91] or cell wall proteins [91] were
562 immobilised onto the wells of a Nunc Maxisorp ELISA plate by incubation at 4 °C for 16 h. Remaining
563 binding sites were blocked with PBS containing 3 % bovine serum albumin before addition of the
564 eluted antibody samples (200 µl). Wells containing eluted antibodies were incubated at ambient
565 temperature for 1 h, washed three times with PBS and then 200 µl PBS containing anti-human
566 antibodies conjugated to alkaline phosphatase (Abcam, 1:2000 dilution) was added for 1 h. Wells
567 were again washed three times with PBS and bound alkaline phosphatase quantified using a p-
568 Nitrophenol phosphate substrate solution for ELISA (Merck) and A_{405} readings.

569

570 **Statistical analyses**

571 CFU counts were $\log_{10}$ transformed and displayed as the geometric mean ± geometric standard
572 deviations [95]. Other data are displayed as the mean ± standard deviation or median ± 95 % CI.
573 For all experiments, three or more independent replicates were performed as indicated by individual
574 data points. Data were analysed by one-way ANOVA, two-way ANOVA, or Kruskal Wallis, with
575 appropriate post-hoc multiple comparison test as detailed in figure legends using GraphPad Prism
576 (V8.0).

577

Acknowledgements

We thank the blood donors, without whom this study would not have been possible. Joan Geoghegan (University of Birmingham) and Angelika Grundling (Imperial College London) are thanked for providing strains. EVKL was supported by a Wellcome Trust PhD Studentship (203812/Z/16/Z). We acknowledge the technical support and the use of equipment from the SAFB Flow Cytometry Facility (Imperial College London). AME acknowledges funding from the Rosetrees Trust and from the Imperial NIHR Biomedical Research Centre, Imperial College London. All authors acknowledge the provision of strains by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) Program: under NIAID/ NIH Contract No. HHSN272200700055C.

Contributions and study design

EVKL and AME designed the experiments, conducted the experiments, analyzed the data, and wrote the manuscript. The funders had no role in the study design, interpretation of the findings or the writing of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

All data supporting the findings of this study are available within the paper and its Supplementary Information.

References

1. **Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr.** *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 28(3):603-61. 2015.
2. **Yang Z, Wang J, Wang W, Zhang Y, Han L, Zhang Y, Nie X, Zhan S.** Proportions of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in patients with surgical site infections in mainland China: a systematic review and meta-analysis. *PLoS One*. 10(1):e0116079. 2015.
3. **Kuehl R, Morata L, Boeing C, Subirana I, Seifert H, Rieg S, Kern WV, Kim HB, Kim ES, Liao CH, Tilley R, Lopez-Cortés LE, Llewelyn MJ, Fowler VG, Thwaites G, Cisneros JM, Scarborough M, Nsutebu E, Gurgui Ferrer M, Pérez JL, Barlow G, Hopkins S, Ternavasio-de la Vega HG, Török ME, Wilson P, Kaasch AJ, Soriano A; International *Staphylococcus aureus* collaboration study group and the ESCMID Study Group for Bloodstream Infections,**

613 **Endocarditis and Sepsis.** Defining persistent *Staphylococcus aureus* bacteraemia: secondary
614 analysis of a prospective cohort study. *Lancet Infect Dis*. 20(12):1409-1417. 2020.
615 4. **Bergin SP, Holland TL, Fowler VG Jr, Tong SYC.** Bacteremia, Sepsis, and Infective
616 Endocarditis Associated with *Staphylococcus aureus*. *Curr Top Microbiol Immunol*.409:263-
617 296. 2017.
618 5. **Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, Cheng AC, Dudley T,**
619 **Oddone EZ.** Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Arch Intern*
620 *Med*. 22;163(17):2066-72. 2003.
621 6. **Nguyen TH, Cheung GYC, Rigby KM, Kamenyeva O, Kabat J, Sturdevant DE, Villaruz AE, Liu**
622 **R, Piewngam P, Porter AR, Firdous S, Chiou J, Park MD, Hunt RL, Almufarriji FMF, Tan VY,**
623 **Asiamah TK, McCausland JW, Fisher EL, Yeh AJ, Bae JS, Kobayashi SD, Wang JM, Barber DL,**
624 **DeLeo FR, Otto M.** Rapid pathogen-specific recruitment of immune effector cells in the skin
625 by secreted toxins. *Nat Microbiol*. 7(1):62-72. 2022.
626 7. **DeLeo FR, Diep BA, Otto M.** Host defense and pathogenesis in *Staphylococcus aureus*
627 infections. *Infect Dis Clin North Am*. 23(1):17-34. 2009.
628 8. **Ellson CD, Davidson K, Ferguson GJ, O'Connor R, Stephens LR, Hawkins PT.** Neutrophils
629 from p40phox-/- mice exhibit severe defects in NADPH oxidase regulation and oxidant-
630 dependent bacterial killing. *J Exp Med*. 203(8):1927-37. 2006.
631 9. **Ha KP, Clarke RS, Kim GL, Brittan JL, Rowley JE, Mavridou DAI, Parker D, Clarke TB, Nobbs**
632 **AH, Edwards AM.** Staphylococcal DNA Repair Is Required for Infection. *mBio*. 11(6):e02288-
633 20. 2020.
634 10. **Scribner DJ, Fahrney D.** Neutrophil receptors for IgG and complement: their roles in the
635 attachment and ingestion phases of phagocytosis. *J Immunol*. 116(4):892-7. 1976.
636 11. **Wright AE, Douglas SR.** An experimental investigation of the rôle of the blood fluids in
637 connection with phagocytosis. *Proc R Soc Lond*. 72357–370. 1904.
638 12. **Leuzzi R, Bodini M, Thomsen IP, Soldaini E, Bartolini E, Muzzi A, Clemente B, Galletti B,**
639 **Manetti AGO, Giovani C, Censini S, Budroni S, Spensieri F, Borgogni E, Rossi Paccani S,**
640 **Margarit I, Bagnoli F, Giudice GD, Creech CB.** Dissecting the Human Response to
641 *Staphylococcus aureus* Systemic Infections. *Front Immunol*. 12:749432. 2021.
642 13. **Guerra FE, Borgogna TR, Patel DM, Sward EW, Voyich JM.** Epic Immune Battles of History:
643 Neutrophils vs. *Staphylococcus aureus*. *Front Cell Infect Microbiol*. 7:286. 2017.
644 14. **Holtfreter S, Kolata J, Bröker BM.** Towards the immune proteome of *Staphylococcus aureus*
645 - The anti-*S. aureus* antibody response. *Int J Med Microbiol*. 300(2-3):176-92. 2010.
646 15. **Dryla A, Prustomersky S, Gelbmann D, Hanner M, Bettinger E, Kocsis B, Kustos T, Henics T,**
647 **Meinke A, Nagy E.** Comparison of antibody repertoires against *Staphylococcus aureus* in
648 healthy individuals and in acutely infected patients. *Clin Diagn Lab Immunol*. 12(3):387-98.
649 2005.
650 16. **Wergeland HI, Haaheim LR, Natås OB, Wesenberg F, Oeding P.** Antibodies to staphylococcal
651 peptidoglycan and its peptide epitopes, teichoic acid, and lipoteichoic acid in sera from
652 blood donors and patients with staphylococcal infections. *J Clin Microbiol*. 27(6):1286-91.
653 1989.
654 17. **Kim MJ, Rah SY, An JH, Kurokawa K, Kim UH, Lee BL.** Human anti-peptidoglycan-IgG-
655 mediated opsonophagocytosis is controlled by calcium mobilization in phorbol myristate
656 acetate-treated U937 cells. *BMB Rep*. 48(1):36-41. 2015.
657 18. **Jung DJ, An JH, Kurokawa K, Jung YC, Kim MJ, Aoyagi Y, Matsushita M, Takahashi S, Lee HS,**
658 **Takahashi K, Lee BL.** Specific serum Ig recognizing staphylococcal wall teichoic acid induces
659 complement-mediated opsonophagocytosis against *Staphylococcus aureus*. *J Immunol*.
660 189(10):4951-9. 2012.
661 19. **Lee JH, Kim NH, Winstel V, Kurokawa K, Larsen J, An JH, Khan A, Seong MY, Lee MJ,**
662 **Andersen PS, Peschel A, Lee BL.** Surface Glycopolymers Are Crucial for In Vitro Anti-Wall

663 Teichoic Acid IgG-Mediated Complement Activation and Opsonophagocytosis of
664 *Staphylococcus aureus*. *Infect Immun*. 83(11):4247-55. 2015.
665 20. **Theilacker C, Kropec A, Hammer F, Sava I, Wobser D, Sakinc T, Codée JD, Hogendorf WF,**
666 **van der Marel GA, Huebner J.** Protection against *Staphylococcus aureus* by antibody to the
667 polyglycerolphosphate backbone of heterologous lipoteichoic acid. *J Infect Dis*. 205(7):1076-
668 85. 2012.
669 21. **de Jong NWM, van Kessel KPM, van Strijp JAG.** Immune Evasion by *Staphylococcus aureus*.
670 *Microbiol Spectr*. 7(2). 2019.
671 22. **Thammavongsa V, Kim HK, Missiakas D, Schneewind O.** Staphylococcal manipulation of
672 host immune responses. *Nat Rev Microbiol*. 13(9):529-43. 2015.
673 23. **Cruz AR, Boer MAD, Strasser J, Zwarthoff SA, Beurskens FJ, de Haas CJC, Aerts PC, Wang G,**
674 **de Jong RN, Bagnoli F, van Strijp JAG, van Kessel KPM, Schuurman J, Preiner J, Heck AJR,**
675 **Rooijakkers SHM.** Staphylococcal protein A inhibits complement activation by interfering
676 with IgG hexamer formation. *Proc Natl Acad Sci U S A*. 118(7):e2016772118. 2021.
677 24. **Falugi F, Kim HK, Missiakas DM, Schneewind O.** Role of protein A in the evasion of host
678 adaptive immune responses by *Staphylococcus aureus*. *mBio*. 4(5):e00575-13. 2013.
679 25. **Zhang L, Jacobsson K, Vasi J, Lindberg M, Frykberg L.** A second IgG-binding protein in
680 *Staphylococcus aureus*. *Microbiology*. 144(4):985-991. 1998.
681 26. **Smith EJ, Visai L, Kerrigan SW, Speziale P, Foster TJ.** The Sbi protein is a multifunctional
682 immune evasion factor of *Staphylococcus aureus*. *Infect Immun*. 79(9):3801-9. 2011.
683 27. **de Haas CJ, Veldkamp KE, Peschel A, Weerkamp F, Van Wamel WJ, Heezius EC, Poppelier**
684 **MJ, Van Kessel KP, van Strijp JA.** Chemotaxis inhibitory protein of Staphylococcus aureus, a
685 bacterial antiinflammatory agent. *J Exp Med*. 199(5):687-95. 2004.
686 28. **Ko YP, Kuipers A, Freitag CM, Jongerius I, Medina E, van Rooijen WJ, Spaan AN, van Kessel**
687 **KP, Höök M, Rooijakkers SH.** Phagocytosis escape by a *Staphylococcus aureus* protein that
688 connects complement and coagulation proteins at the bacterial surface. *PLoS Pathog*.
689 9(12):e1003816. 2013.
690 29. **Rooijakkers SH, Ruyken M, Roos A, Daha MR, Presanis JS, Sim RB, van Wamel WJ, van**
691 **Kessel KP, van Strijp JA.** Immune evasion by a staphylococcal complement inhibitor that acts
692 on C3 convertases. *Nat Immunol*. 6(9):920-7. 2005.
693 30. **Rooijakkers SH, Ruyken M, van Roon J, van Kessel KP, van Strijp JA, van Wamel WJ.** Early
694 expression of SCIN and CHIPS drives instant immune evasion by *Staphylococcus aureus*. *Cell*
695 *Microbiol*. 8(8):1282-93. 2006.
696 31. **Howden BP, Giulieri SG, Wong Fok Lung T, Baines SL, Sharkey LK, Lee JYH, Hachani A, Monk**
697 **IR, Stinear TP.** *Staphylococcus aureus* host interactions and adaptation. *Nat Rev Microbiol*. 1-
698 16. 2023.
699 32. **Pidgeon SE, Pires MM.** Cell Wall Remodeling of *Staphylococcus aureus* in Live
700 *Caenorhabditis elegans*. *Bioconjug Chem*. 28(9):2310-2315. 2017.
701 33. **Sobral R, Tomasz A.** The Staphylococcal Cell Wall. *Microbiol Spectr*. 7(4). 2019.
702 34. **Salamaga B, Kong L, Pasquina-Lemonche L, Lafage L, von Und Zur Muhlen M, Gibson JF,**
703 **Grybchuk D, Tooke AK, Panchal V, Culp EJ, Tatham E, O'Kane ME, Catley TE, Renshaw SA,**
704 **Wright GD, Plevka P, Bullough PA, Han A, Hobbs JK, Foster SJ.** Demonstration of the role of
705 cell wall homeostasis in *Staphylococcus aureus* growth and the action of bactericidal
706 antibiotics. *Proc Natl Acad Sci U S A*. 118(44):e2106022118. 2021.
707 35. **Monteiro JM, Fernandes PB, Vaz F, Pereira AR, Tavares AC, Ferreira MT, Pereira PM, Veiga**
708 **H, Kuru E, VanNieuwenhze MS, Brun YV, Filipe SR, Pinho MG.** Cell shape dynamics during
709 the staphylococcal cell cycle. *Nat Commun*. 6:8055. 2015.
710 36. **Sutton JAF, Carnell OT, Lafage L, Gray J, Biboy J, Gibson JF, Pollitt EJG, Tazoll SC, Turnbull**
711 **W, Hajdamowicz NH, Salamaga B, Pidwill GR, Condliffe AM, Renshaw SA, Vollmer W,**
712 **Foster SJ.** *Staphylococcus aureus* cell wall structure and dynamics during host-pathogen
713 interaction. *PLoS Pathog*. 17(3):e1009468. 2021.

714 37. **Ledger EVK, Mesnage S, Edwards AM.** Human serum triggers antibiotic tolerance in
715 *Staphylococcus aureus. Nat Commun*. 13(1):2041. 2022.
716 38. **Ellington JK, Harris M, Hudson MC, Vishin S, Webb LX, Sherertz R.** Intracellular
717 Staphylococcus aureus and antibiotic resistance: implications for treatment of
718 staphylococcal osteomyelitis. *J Orthop Res*. 24(1):87-93. 2006.
719 39. **Raineri EJM, Yedavally H, Salvati A, van Dijl JM.** Time-resolved analysis of *Staphylococcus*
720 *aureus* invading the endothelial barrier. Virulence. 11(1):1623-1639. 2020.
721 40. **Reed P, Atilano ML, Alves R, Hoiczyk E, Sher X, Reichmann NT, Pereira PM, Roemer T, Filipe**
722 **SR, Pereira-Leal JB, Ligoxygakis P, Pinho MG.** *Staphylococcus aureus* Survives with a Minimal
723 Peptidoglycan Synthesis Machine but Sacrifices Virulence and Antibiotic Resistance. *PLoS*
724 *Pathog*. 11(5):e1004891. 2015.
725 41. **Hines KM, Alvarado G, Chen X, Gatto C, Pokorny A, Alonzo F 3rd, Wilkinson BJ, Xu L.**
726 Lipidomic and Ultrastructural Characterization of the Cell Envelope of *Staphylococcus aureus*
727 Grown in the Presence of Human Serum. *mSphere*. 5(3):e00339-20. 2020.
728 42. **Yarwood JM, McCormick JK, Paustian ML, Kapur V, Schlievert PM.** Repression of the
729 *Staphylococcus aureus* accessory gene regulator in serum and *in vivo*. *J Bacteriol*.
730 184(4):1095-101. 2002.
731 43. **James EH, Edwards AM, Wigneshweraraj S.** Transcriptional downregulation of agr
732 expression in *Staphylococcus aureus* during growth in human serum can be overcome by
733 constitutively active mutant forms of the sensor kinase AgrC. *FEMS Microbiol Lett*.
734 349(2):153-62. 2013.
735 44. **Manifold-Wheeler BC, Elmore BO, Triplett KD, Castleman MJ, Otto M, Hall PR.** Serum
736 Lipoproteins Are Critical for Pulmonary Innate Defense against *Staphylococcus aureus*
737 Quorum Sensing. *J Immunol*. 196(1):328-35. 2016.
738 45. **Peterson MM, Mack JL, Hall PR, Alsup AA, Alexander SM, Sully EK, Sawires YS, Cheung AL,**
739 **Otto M, Gresham HD.** Apolipoprotein B Is an innate barrier against invasive *Staphylococcus*
740 *aureus* infection. *Cell Host Microbe*. 4(6):555-66. 2008.
741 46. **Hall PR, Elmore BO, Spang CH, Alexander SM, Manifold-Wheeler BC, Castleman MJ, Daly**
742 **SM, Peterson MM, Sully EK, Femling JK, Otto M, Horswill AR, Timmins GS, Gresham HD.**
743 Nox2 modification of LDL is essential for optimal apolipoprotein B-mediated control of agr
744 type III *Staphylococcus aureus* quorum-sensing. *PLoS Pathog*. 9(2):e1003166. 2013.
745 47. **Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, Braughton KR,**
746 **Shabb DW, Diep BA, Chambers HF, Otto M, DeLeo FR.** Global changes in *Staphylococcus*
747 *aureus* gene expression in human blood. *PLoS One*. 6(4):e18617. 2011.
748 48. **Cybulska J, Jeljaszewicz J.** Bacteriostatic activity of serum against staphylococci. *J Bacteriol*.
749 91(3):953-62. 1966.
750 49. **Ehrenkranz NJ, Elliott DF, Zarco R.** Serum Bacteriostasis of *Staphylococcus aureus. Infect*
751 *Immun*. 3(5):664-70. 1971.
752 50. **Painter KL, Hall A, Ha KP, Edwards AM.** The Electron Transport Chain Sensitizes
753 *Staphylococcus aureus* and *Enterococcus faecalis* to the Oxidative Burst. *Infect Immun*.
754 85(12):e00659-17. 2017.
755 51. **Ha KP, Clarke RS, Kim GL, Brittan JL, Rowley JE, Mavridou DAI, Parker D, Clarke TB, Nobbs**
756 **AH, Edwards AM.** Staphylococcal DNA Repair Is Required for Infection. *mBio*. 11(6):e02288-
757 20. 2020.
758 52. **Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, Bayles KW.** A genetic resource
759 for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus*
760 genes. *mBio*. 4(1):e00537-12. 2013.
761 53. **Horsburgh MJ, Aish JL, White IJ, Shaw L, Lithgow JK, Foster SJ.** sigmaB modulates virulence
762 determinant expression and stress resistance: characterization of a functional *rsbU* strain
763 derived from *Staphylococcus aureus* 8325-4. *J Bacteriol*. 184(19):5457-67. 2002.

764 54. **Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF,**
765 **Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft**
766 **DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J,**
767 **Tran K, Kang K, Hance IR, Nelson KE, Fraser CM.** Insights on evolution of virulence and
768 resistance from the complete genome analysis of an early methicillin-resistant
769 *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus*
770 *epidermidis* strain. *J Bacteriol*. 2005 187(7):2426-38. 2005.
771 55. **Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K.** Genome sequence of
772 *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes:
773 polymorphism and evolution of two major pathogenicity islands. *J Bacteriol*. 190(1):300-10.
774 2008.
775 56. **Painter KL, Hall A, Ha KP, Edwards AM.** The Electron Transport Chain Sensitizes
776 *Staphylococcus aureus* and *Enterococcus faecalis* to the Oxidative Burst. *Infect Immun*.
777 85(12):e00659-17. 2017.
778 57. **Nagl M, Kacani L, Müllauer B, Lemberger EM, Stoiber H, Sprinzl GM, Schennach H, Dierich**
779 **MP.** Phagocytosis and killing of bacteria by professional phagocytes and dendritic cells. *Clin*
780 *Diagn Lab Immunol*. 9(6):1165-8. 2002.
781 58. **Wonfor T, Li S, Dunphy RW, Macpherson A, van den Elsen J, Laabei M.** Novel method for
782 detecting complement C3 deposition on *Staphylococcus aureus*. *Sci Rep*. 12(1):15766. 2022.
783 59. **Boero E, Brinkman I, Juliet T, van Yperen E, van Strijp JAG, Rooijakkers SHM, van Kessel**
784 **KPM.** Use of Flow Cytometry to Evaluate Phagocytosis of *Staphylococcus aureus* by Human
785 Neutrophils. *Front Immunol*. 12:635825. 2021.
786 60. **Silver LL.** Fosfomycin: Mechanism and Resistance. *Cold Spring Harb Perspect Med*.
787 7:a025262. 2017.
788 61. **Pujol M, Miró JM, Shaw E, Aguado JM, San-Juan R, Puig-Asensio M, Pigrau C, Calbo E,**
789 **Montejo M, Rodriguez-Álvarez R, Garcia-Pais MJ, Pintado V, Escudero-Sánchez R, Lopez-**
790 **Contreras J, Morata L, Montero M, Andrés M, Pasquau J, Arenas MD, Padilla B, Murillas J,**
791 **Jover-Sáenz A, López-Cortes LE, García-Pardo G, Gasch O, Videla S, Hereu P, Tebé C,**
792 **Pallarès N, Sanllorente M, Domínguez MÁ, Càmara J, Ferrer A, Padullés A, Cuervo G,**
793 **Carratalà J; MRSA Bacteremia (BACSARM) Trial Investigators.** Daptomycin Plus Fosfomycin
794 Versus Daptomycin Alone for Methicillin-resistant *Staphylococcus aureus* Bacteremia and
795 Endocarditis: A Randomized Clinical Trial. *Clin Infect Dis*. 72(9):1517-1525. 2021.
796 62. **Wright AJ, Wilkowske CJ.** The penicillins. *Mayo Clin Proc*. 66(10):1047-63. 1991.
797 63. **Karlowsky JA, Kaplan N, Hafkin B, Hoban DJ, Zhanel GG.** AFN-1252, a FabI inhibitor,
798 demonstrates a *Staphylococcus*-specific spectrum of activity. *Antimicrob Agents Chemother*.
799 53(8):3544-8. 2009.
800 64. **Sanders CC.** Ciprofloxacin: in vitro activity, mechanism of action, and resistance. *Rev Infect*
801 *Dis*. 10(3):516-27. 1988.
802 65. **Douglas EJA, Palk N, Brignoli T, Altwiley D, Boura M, Laabei M, Recker M, Cheung GCY, Liu**
803 **R, Hseih RC, Otto M, O'Brien E, McLoughlin RM, Massey RC.** Extensive remodelling of the
804 cell wall during the development of *Staphylococcus aureus* bacteraemia. *Elife*. 12:RP87026.
805 2023.
806 66. **Rajagopal M, Walker S.** Envelope Structures of Gram-Positive Bacteria. *Curr Top Microbiol*
807 *Immunol*. 404:1-44. 2017.
808 67. **Gautam S, Kim T, Lester E, Deep D, Spiegel DA.** Wall teichoic acids prevent antibody binding
809 to epitopes within the cell wall of *Staphylococcus aureus*. *ACS Chem Biol*. 11(1):25-30. 2016.
810 68. **Leonard AC, Goncheva MI, Gilbert SE, Shareefdeen H, Petrie LE, Thompson LK, Khursigara**
811 **CM, Heinrichs DE, Cox G.** Autolysin-mediated peptidoglycan hydrolysis is required for the
812 surface display of *Staphylococcus aureus* cell wall-anchored proteins. *Proc Natl Acad Sci U S*
813 *A*. 120(12):e2301414120. 2023.

814 69. **Miller LS, Fowler VG, Shukla SK, Rose WE, Proctor RA.** Development of a vaccine against
815 *Staphylococcus aureus* invasive infections: Evidence based on human immunity, genetics and
816 bacterial evasion mechanisms. *FEMS Microbiol Rev*. 44(1):123-153. 2020.
817 70. **Tsai CM, Caldera JR, Hajam IA, Chiang AWT, Tsai CH, Li H, Díez ML, Gonzalez C, Trieu D,**
818 **Martins GA, Underhill DM, Arditi M, Lewis NE, Liu GY.** Non-protective immune imprint
819 underlies failure of *Staphylococcus aureus* IsdB vaccine. *Cell Host Microbe*. 30(8):1163-
820 1172.e6. 2022.
821 71. **Falcón R, Martínez A, Albert E, Madrid S, Oltra R, Giménez E, Soriano M, Vinuesa V,**
822 **Gozalbo D, Gil ML, Navarro D.** High vancomycin MICs within the susceptible range in
823 *Staphylococcus aureus* bacteraemia isolates are associated with increased cell wall thickness
824 and reduced intracellular killing by human phagocytes. *Int J Antimicrob Agents*. 47(5):343-50.
825 2016.
826 72. **Tickle ARH, Ledger EVK, Edwards AM.** Human serum induces daptomycin tolerance in
827 *Enterococcus faecalis* and viridans group streptococci. *Microbiology*. 168(12). 2022.
828 73. **Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, Braughton KR,**
829 **Shabb DW, Diep BA, Chambers HF, Otto M, DeLeo FR.** Global changes in *Staphylococcus*
830 *aureus* gene expression in human blood. *PLoS One*. 6(4):e18617. 2011.
831 74. **Kim HK, Missiakas D, Schneewind O.** Mouse models for infectious diseases caused by
832 *Staphylococcus aureus*. *J Immunol Methods*. 410:88-99. 2014.
833 75. **Foster TJ.** Surface Proteins of *Staphylococcus aureus*. *Microbiol Spectr*. 7(4). 2019.
834 76. **Luna BM, Nielsen TB, Cheng B, Pantapalangkoor P, Yan J, Boyle-Vavra S, Bruhn KW,**
835 **Montgomery C, Spellberg B, Daum R.** Vaccines targeting *Staphylococcus aureus* skin and
836 bloodstream infections require different composition. *PLoS One*. 14(6):e0217439. 2019.
837 77. **Clegg J, Soldaini E, McLoughlin RM, Rittenhouse S, Bagnoli F, Phogat S.** *Staphylococcus*
838 *aureus* Vaccine Research and Development: The Past, Present and Future, Including Novel
839 Therapeutic Strategies. *Front Immunol*. 12:705360. 2021.
840 78. **Marrie TJ, Costerton JW.** Scanning and transmission electron microscopy of in situ bacterial
841 colonization of intravenous and intraarterial catheters. *J Clin Microbiol*. 19(5):687-93. 1984.
842 79. **Hetem DJ, de Ruiter SC, Buiting AGM, Kluytmans JAJW, Thijsen SF, Vlaminckx BJM,**
843 **Wintermans RGF, Bonten MJM, Ekkelenkamp MB.** Preventing *Staphylococcus aureus*
844 bacteremia and sepsis in patients with Staphylococcus aureus colonization of intravascular
845 catheters: a retrospective multicenter study and meta-analysis. *Medicine*. 90(4):284-288.
846 2011.
847 80. **Minejima E, Mai N, Bui N, Mert M, Mack WJ, She RC, Nieberg P, Spellberg B, Wong-**
848 **Beringer A.** Defining the Breakpoint Duration of *Staphylococcus aureus* Bacteremia
849 Predictive of Poor Outcomes. *Clin Infect Dis*. 70(4):566-573. 2020.
850 81. **Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, Leung DY.**
851 Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med*.
852 347(15):1151-60. 2002.
853 82. **Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino**
854 **J, Huttner K, Gallo RL**. Innate antimicrobial peptide protects the skin from invasive bacterial
855 infection. *Nature*. 414(6862):454-7. 2001.
856 83. **Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B,**
857 **Gudmundsson GH, Gallo RL.** Cutaneous injury induces the release of cathelicidin
858 antimicrobial peptides active against group A *Streptococcus*. *J. Invest. Dermatol.* **117**, 91–97
859 (2001).
860 84. **Burian M, Plange J, Schmitt L, Kaschke A, Marquardt Y, Huth L, Baron JM, Hornef MW,**
861 **Wolz C, Yazdi AS.** Adaptation of *Staphylococcus aureus* to the Human Skin Environment
862 Identified Using an *ex vivo* Tissue Model. *Front Microbiol*. 12:728989. 2021.

863 85. **Kraus D, Herbert S, Kristian SA, Khosravi A, Nizet V, Götz F, Peschel A.** The GraRS regulatory
864 system controls *Staphylococcus aureus* susceptibility to antimicrobial host defenses. *BMC*
865 *Microbiol*. 8:85. 2008.
866 86. **Cheung AL, Cho J, Bayer AS, Yeaman MR, Xiong YQ, Donegan NP, Mikheyeva IV, Lee GY,**
867 **Yang SJ.** Role of the *Staphylococcus aureus* Extracellular Loop of GraS in Resistance to
868 Distinct Human Defense Peptides in PMN and Invasive Cardiovascular infections. *Infect*
869 *Immun*. 89(10):e0034721. 2021.
870 87. **Friberg C, Haaber JK, Vestergaard M, Fait A, Perrot V, Levin BR, Ingmer H.** Human
871 antimicrobial peptide, LL-37, induces non-inheritable reduced susceptibility to vancomycin in
872 *Staphylococcus aureus*. *Sci Rep*. 10(1):13121. 2020.
873 88. **Ranganathan N, Johnson R, Edwards AM.** The general stress response of *Staphylococcus*
874 *aureus* promotes tolerance of antibiotics and survival in whole human blood. *Microbiology*.
875 166(11):1088-1094. 2020.
876 89. **Rowe SE, Wagner NJ, Li L, Beam JE, Wilkinson AD, Radlinski LC, Zhang Q, Miao EA, Conlon**
877 **BP.** Reactive oxygen species induce antibiotic tolerance during systemic *Staphylococcus*
878 *aureus* infection. *Nat Microbiol*. 5(2):282-290. 2020.
879 90. **Beam JE, Rowe SE, Conlon BP.** Shooting yourself in the foot: How immune cells induce
880 antibiotic tolerance in microbial pathogens. *PLoS Pathog*. 17(7):e1009660. 2021.
881 91. **Shen F, Tang X, Cheng W, Wang Y, Wang C, Shi X, An Y, Zhang Q, Liu M, Liu B, Yu L.**
882 Fosfomycin enhances phagocyte-mediated killing of *Staphylococcus aureus* by extracellular
883 traps and reactive oxygen species. *Sci Rep*. 6:19262. 2016.
884 92. **Berti A, Rose W, Nizet V, Sakoulas G.** Antibiotics and Innate Immunity: A Cooperative Effort
885 Toward the Successful Treatment of Infections. *Open Forum Infect Dis*. 7(8):ofaa302. 2020.
886 93. **Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, Bayles KW.** A genetic resource
887 for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus*
888 genes. *mBio*. 4(1):e00537-12. 2013.
889 94. **Higgins J., Loughman A., van Kessel K. P., van Strijp J. A., Foster T. J.** Clumping factor A of
890 *Staphylococcus aureus* inhibits phagocytosis by human polymorphonuclear leucocytes. *FEMS*
891 *Microbiol. Lett*. 258:290–296. 2006.
892 95. **Olsen CH**. Review of the use of statistics in infection and immunity. *Infect Immun*.
893 1(12):6689-92. 2003.
894 96. **Cunnion KM, Hair PS, Buescher ES.** Cleavage of complement C3b to iC3b on the surface of
895 *Staphylococcus aureus* is mediated by serum complement factor I. *Infect Immun*. 72(5):2858-
896 63. 2004.
897 97. **Easmon CS, Lanyon H, Cole PJ.** Use of lysostaphin to remove cell-adherent staphylococci
898 during *in vitro* assays of phagocyte function. *Br J Exp Pathol*. 59(4):381-5. 1978.
899 98. **Simon GL, Miller HG, Borenstein DG.** Synovial fluid inhibits killing of *Staphylococcus aureus*
900 by neutrophils. *Infect Immun*. 40(3):1004-10. 1983.
901 99. **Schneewind O, Mihaylova-Petkov D, Model P.** Cell wall sorting signals in surface proteins of
902 gram-positive bacteria. *EMBO J*. 12(12):4803-11. 1993.
903
904
905