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Analysis of the dynamics of *Staphylococcus aureus* binding to white blood cells using whole blood assay and geno-to-pheno mapping

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ABSTRACT

Given that binding and internalization of bacteria to host cells promotes infections and invasion, we aimed at characterizing how various *S. aureus* isolates adhere to and are internalized by different white blood cells. In particular, the role of genetic determinants on the association kinetics should be unveiled. A flow cytometric (FACS) whole blood assay with fluorescently labelled isolates was applied to 56 clinical *S. aureus* isolates. This phenotypic data was then linked to previously obtained genotyping data (334 genes) with the help of a re-description mining algorithm. Professional phagocytes showed a time-dependent increase of bacterial adhesion and internalization. Isolates showing higher affinity to granulocytes were associated with lower binding to monocytes. In contrast binding activity between *S. aureus* and lymphocytes could be subdivided into two phases. Preliminary binding (phase 1) was highest directly after co-incubation and was followed by *S. aureus* detachment or by sustained binding of a small lymphocyte subset (phase 2). Strain-dependent low granulocyte binding was observed for clonal complex 5 (CC5) isolates (MRSA), as compared to CC30 and CC45 (MSSA). *S. aureus* isolates associated with low granulocyte phagocytosis were characterized by the presence (*cap8*, *can*) and the absence (*sasG*, *lukD*, *isdA*, *splA*, *setC*) of specific hybridization signals.

1. Introduction

Staphylococcus aureus is a major human pathogen causing acute, chronic and recurrent infections (Lowy, 1998). At a rate of approximately 25/100.000 population (Laupland, 2013) *S. aureus* is also a major cause of blood stream infections, with an ensuing death toll comparable to certain types of malignant diseases (Tom et al., 2014). Contributing pathogenic mechanisms are concerted by a fine-tuned interplay of metabolic adaptation, gene regulation, virulence factor expression, and host defense which have been characterized in great detail but by no means exhaustively due to the complexity of interactions (Conlon et al., 2016; Horn et al. 2018; Tuchscherr et al., 2015; Laabei and Massey, 2016; Dastgheyb and Otto, 2015).

The binding and uptake of *S. aureus* by blood cells, in particular by phagocytes competent for bacterial killing, is a cornerstone of the innate anti-staphylococcal immunity. This is exemplified by chronic granulomatous disease characterized by impaired intracellular killing (Mackay et al., 2000). This interaction of *S. aureus* with blood cells is a multifactorial process; it may involve classic opsonin-mediated phagocytosis but it may also be based on or enhanced by host cell recognition of bacterial surface molecules such as the MSCRAMM (Foster et al., 2014) or soluble molecules of the SERAM family (Gresham et al., 2000). Feil et al. (2003) highlighted that despite detailed characterization of putative virulence factors in the *S. aureus* genome, the differences in pathogenic potential of clinical isolates remain largely unknown due to complex regulatory and epistatic processes.

The intracellular compartment of most eukaryotic cells is well protected as an immune privileged site and may offer shelter against the action of most antimicrobial drugs (Löffler et al., 2014). Experimental studies have documented prolonged bacterial survival within professional phagocytes such as neutrophils or human monocyte derived macrophages (Kubica et al., 2008; Voyich et al., 2005; Gresham et al., 2000). Thus, blood cells may also serve as a shuttle system allowing for unrecognized bacterial persistence and spreading, or serving as a reservoir for recurrent infections.

With respect to the Staphylococcus-blood cell interaction dynamics,

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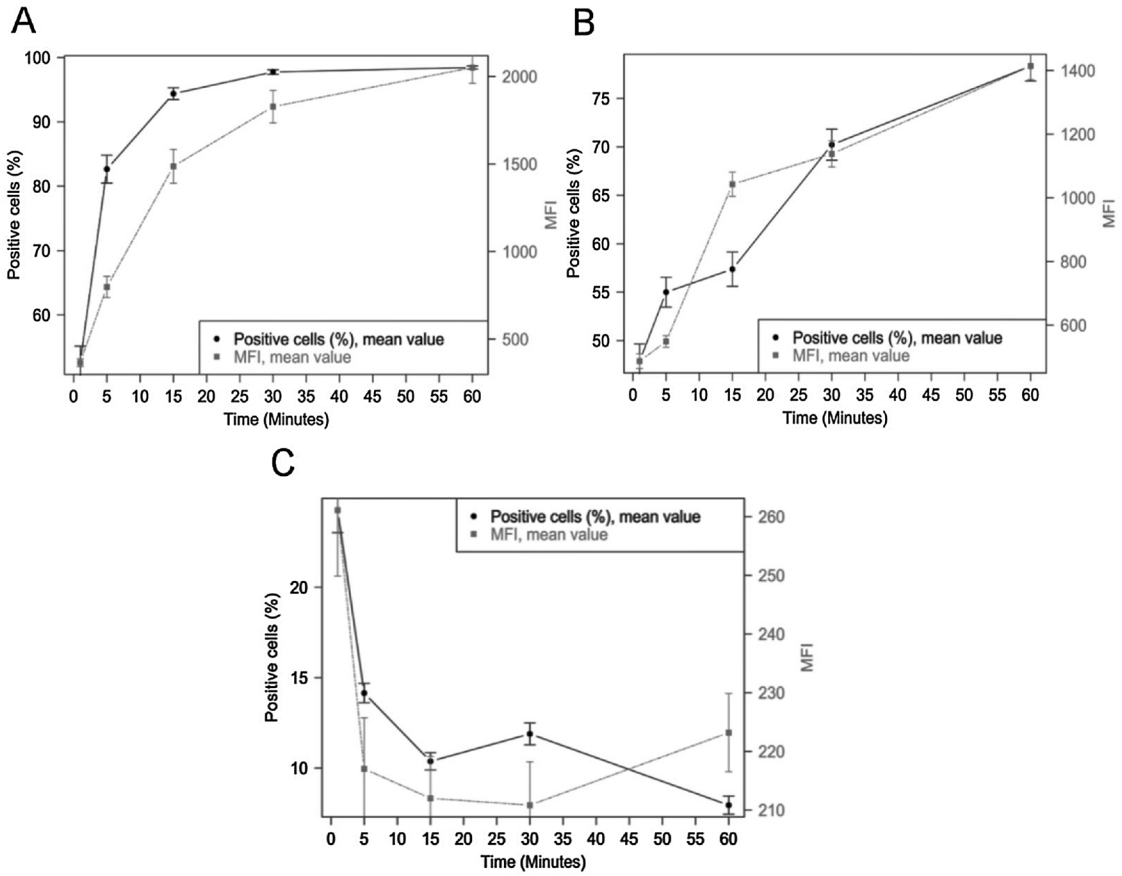


Fig. 1. Binding of fluorescent labeled *S. aureus* isolates to white blood cells during one hour of co-incubation. Fluorescent positive cells (black) and mean fluorescence intensity (MFI) of positive cells (grey) is shown for (**a**) granulocytes, (**b**) monocytes, and (**c**) lymphocytes. Note the different scales on the two y-axes.

we asked i) what are the characteristics of this interaction upon *S. aureus* incubation with the various cell types in a whole blood system, ii) whether certain *S. aureus* clonal clusters (CCs) show distinct dynamic interaction profiles, and iii) whether certain virulence factors (genotype) or major antibiotic resistance traits (MRSA) contribute to differences in the *S. aureus*-host-interaction.

2. Results

2.1. Kinetics of adhesion and invasion of S. aureus to blood cells

For professional phagocytic cells (granulocytes and monocytes) we detected a fast and time-dependent increase in adhesion and internalization of *S. aureus* during one hour of co-incubation with fluorescently labeled bacterial isolates (Fig. 1). The speed of bacterial uptake and the proportion of *S. aureus* positive cells were higher for granulocytes as compared to monocytes. Phagocytic activity was confirmed for the majority of monocytes (> 75 %) while a smaller proportion of cells (< 25 %) did not show *S. aureus* binding within 60 min of co-incubation. Previously, bacterial-lymphocyte interaction was not in the focus of *S. aureus* binding experiments since lymphocytes are a part of specific immunity and not primarily of phagocytosis. Fig. 1C shows that almost 25 % of lymphocytes could bind *S. aureus* directly after co-incubation in whole blood (phase 1, preliminary binding). Yet, the follow-up kinetics revealed that initial binding was not stable (phase 2, detachment or sustained binding).

2.2. Differences between MRSA and MSSA

Among the 56 *S. aureus* isolates studied, 29 were methicillin-resistant (MRSA) and 27 methicillin-susceptible (MSSA). Hierarchical clustering of these isolates was performed based on the kinetics of binding to granulocytes (Fig. 2), monocytes (Fig. S1), and lymphocytes (Fig. S2) and is also profiled by the phylogenetic relationship of the isolates (clonal complexes, CC). The most vivid overlap between clustering by cell-bacteria binding kinetics and antibiotic resistance assignment was found for the interaction with granulocytes (Fig. 2). MSSA isolates were dominant in the clusters of isolates that bound to granulocytes at the highest rate (MFI) and fastest pace (percentage of cells positive to labeled bacteria) (Fig. 2 A, line I, purple; Fig. 2 B, line I, dark blue). In contrast, interaction of MRSA isolates with granulocytes was delayed, withstanding adhesion and internalization by granulocytes for longer times (Fig. 2B, line I, red).

The binding kinetics profiled by bacterial resistance phenotype (Fig. 2, line II and Fig. 3) showed that granulocytes indeed could bind and internalize MSSA more efficiently than MRSA isolates. Furthermore, granulocytes co-incubated with MSSA achieved significantly higher fluorescence values (MFI) at all time points suggesting higher phagocytosis capacity towards MSSA vs. MRSA. In contrast to the preference of granulocytes for MSSA, monocytes bound more strongly to MRSA strains than to MSSA strains (Fig. 3). This also holds true for the early attachment to lymphocytes (1 and 5 min).

2.3. Differences between the predominant clonal complexes (CC)

In the following, we concentrate on the three predominant clonal complexes CC5 (25 isolates), CC45 (13 isolates), CC30 (8 isolates), see Fig. S3 for the details on other CCs. MRSA isolates (low granulocyte binding) belong mainly to CC5, harboring one of the most abundant endemic clones in Germany (Rhein-Hesse-epidemic strain, spa-type t003). As expected, phagocytosis of CC5 isolates by granulocytes was significantly slower than that for MSSA isolates belonging to CC30 and

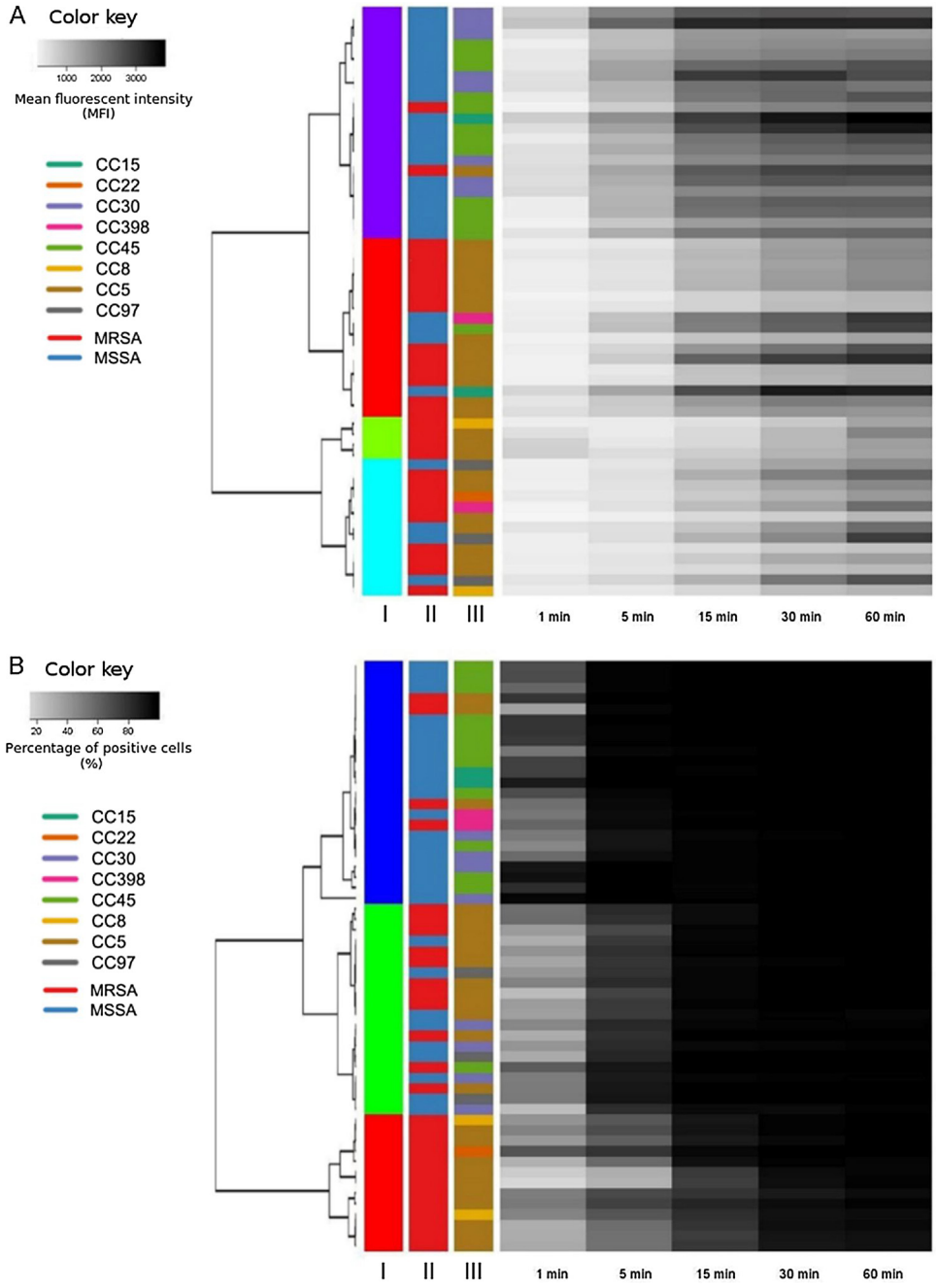


Fig. 2. Functional clustering of *S. aureus* isolates. Clustering is based on the binding of isolates to granulocytes according to (**a**) the mean fluorescence intensity and (**b**) the percentage of granulocytes engaged in contact with *S. aureus*. The three-color bars indicate (I) main functional clusters based on binding kinetics, (II) assignment to MRSA (red) and MSSA (blue) classes, and (III) grouping into clonal complexes.

CC45. We showed that CC5 MRSA strains escape phagocytosis by granulocytes for a longer period of time as compared to other clonal complexes (Fig. 2).

Binding differences between the clonal complexes were most vivid after one minute of co-incubation, when 70 % of granulocytes had initiated contact with CC45, yet only 38 % with CC5 strains (Fig. 4). This suggests that CC5 strains are able to escape phagocytosis by granulocytes longer than strains belonging to other clonal complexes.

Furthermore, we showed that granulocytes phagocytose strains positive for capsular polysaccharide serotype 8 (CP8+) at a faster pace than CP5+ strains (Fig. 5 C). Regarding virulence factors, the presence of *lukD* was associated with lower binding to granulocytes whereas the presence of enterotoxin genes (*egc*) was different between CCs (CC15 and CC97 had no *egc* hybridization signal, see Table S3) but did not show direct association with binding capacity.

2.4. Linking S. aureus genes (genotype) with binding (phenotype)

Redescription mining (Ramakrishnan and Zaki, 2009) is an established approach from the field of data mining. We applied the ReReMi algorithm to the genotypic and phenotypic data of *S. aureus*. Redescription mining offers highly interpretable reasoning describing co-acting sets of objects (Kumar, 2007). As input data, we used both the binarized output of the *S. aureus* DNA hybridization microarray and the real valued output from whole blood-bacteria co-incubation experiments (MFI and the percentage of positive cells). Our aim was to select potential genetic markers correlating with particular behavioral phenotypic patterns for *S. aureus* binding to different white blood cells.

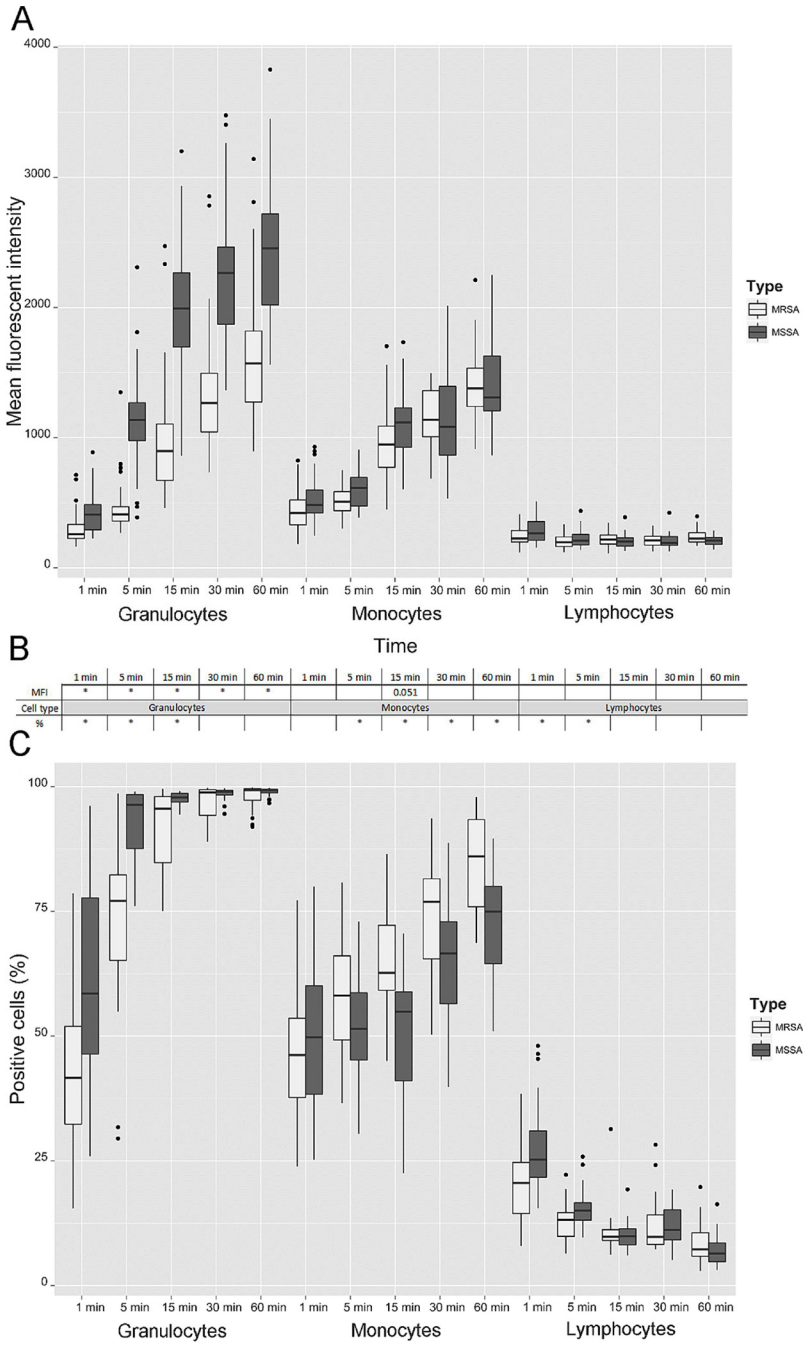


Fig. 3. Kinetics of *S. aureus* binding to white blood cells profiled by methicillin resistance and sensitivity. Panels (**a**) - mean fluorescent intensity and (**c**) - percentage of positive cells show the rates of binding of methicillin-resistant (MRSA) and -susceptible (MSSA) isolates to white blood cells over a span of 60 min at 5 consecutive time points. The statistical significance of the differences between measurements for methicillin-resistant and -susceptible *S. aureus* was determined with the Mann-Whitney-U test (* $p < 0.05$, ** $p < 0.01$), see panel (**b**).

Panels C and D in Fig. 5 show the span of the values measured for isolates in a blood adhesion/internalization experiment together with the logical rules (queries) derived for the *S. aureus* gene set on the DNA microarray. In Table S2 we provide a table of retrieved queries over the experimental outcomes together with their quality measures. Fig. 2 presents the different patterns in the genotypic profile of the isolates under study and eases the reading of the provided redescriptors. Based on the MFI scores for granulocytes incubated with bacteria for 5 min, three groups of logical rules for potentially relevant genes were identified, each logical rule is represented by a specific bar (Fig. 5B). The algorithm found that *S. aureus* strains with positive hybridization signals on the DNA microarray for *capK8*, *cap8* and other capsule 8-realated genes (top right) reached higher MFI values in coupling with granulocytes.

Based on the percentage of *S. aureus* positive granulocytes a similar group of CP8+ strains topped the chart (Fig. 5D) with respect to granulocyte binding. Here, CP8+ strains were followed by CP5+ strains tested positive for a variety of *cap5* genes.

Next, we plotted the presence of automatically selected candidate genes describing particular phenotypes of *S. aureus* bacteria, for MRSA vs. MSSA as well as different clonal complexes 5, 30, and 45 (Fig. 5 A and B). While isolates belonging to CC30 and CC45 (high granulocyte binding) were characterized by capsule 8 (*cap 8*) and collagen binding adhesin (*cna*), the corresponding genes remained undetected for CC5

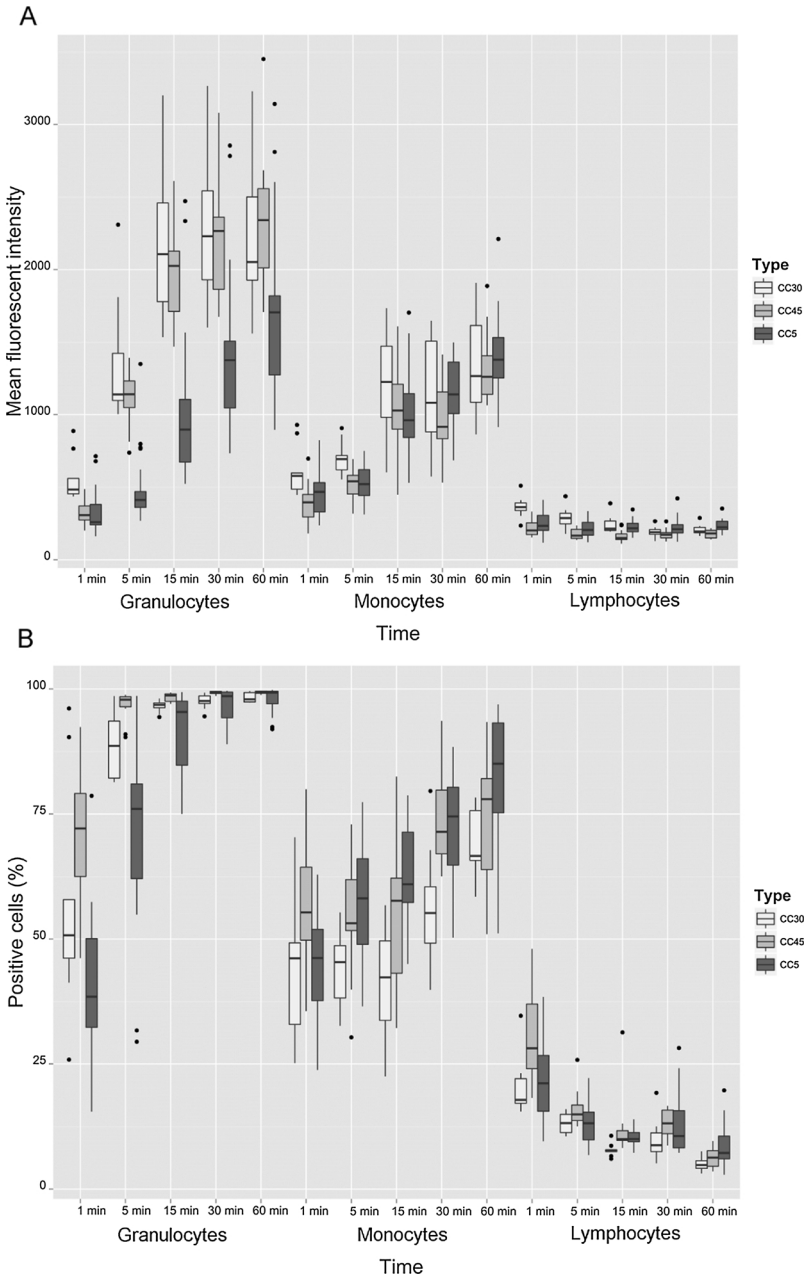


Fig. 4. Kinetics of *S. aureus* binding to white blood cells profiled by clonal complexes (CC). Panels (**a**): mean fluorescent intensity and (b): percentage of positive cells) show the rates of binding and internalization of *S. aureus* isolates from clonal complexes 30 (CC30), CC45, and CC5 to granulocytes, monocytes, and lymphocytes, respectively. Statistical significance of the differences between measurements for distinct *S. aureus* clonal complexes is listed in Table S2.

isolates (low granulocyte binding). The same genetic differences were also found between MRSA (low binding) and MSSA (high binding). While the *egc* operon was uniformly present across CCs 5, 45, and 30, the fibrinogen binding protein coding gene (*fnbB*) was not detected in MSSA CC30.

Better binding of MSSA isolates by granulocytes was associated with characteristic genotypic differences analyzed by DNA microarray (Fig. 5 A), namely the presence of capsule 8 (*cap 8)* and collagen-binding adhesin (*cna)* and the lack of hybridization signals for *S. aureus* surface protein G (*sasG)*, leukocidin D (*lukD)*, transferrin binding protein (*isdA)*, serine protease A (*splA),* and staphylococcal exotoxin C (*setC)*. The majority of genes associated with altered binding to granulocytes are well-known surface proteins (*cna*, *isdA*, *sasG, fnbB)* (Foster et al., 2014). Other candidate genes associated with altered functions, such as leukocidin D (*luk*D), the enterotoxin cluster (*egc),* and a staphylococcal exotoxin-like protein (*setC*) could be related to adhesion and internalization by still unknown mechanisms or by genetic linkage to other more relevant targets of cellular binding. This remains to be confirmed e.g. by investigating isogenic mutants.

3. Discussion

3.1. Summary of principal findings

Staphylococcus aureus is a major pathogen worldwide. Interaction of *S. aureus* with host cells can affect the fate of clinical infections resulting in asymptomatic carriage or invasive infection (Sinha and Fraunholz, 2010; Strobel et al., 2016). For phenotypic analysis, we established a new whole blood flow-cytometric assay (FACS) which allows quantitative analysis of *S. aureus* binding to various white blood cells in the

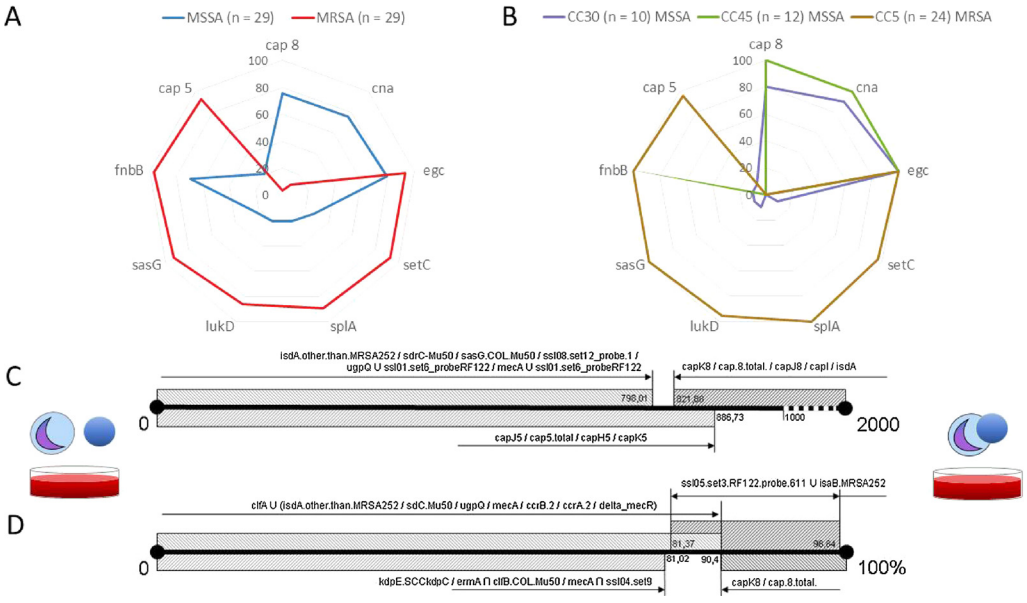


Fig. 5. Panels (**a**) and (**b**) are radar charts for the genes with potential influence on *S. aureus* binding by granulocytes for MRSA vs. MSSA (**a**) and for CCs (**b**). Radii scores from 0 to 100 are related to (**a**) MRSA vs. MSSA with positive hybridization signals to a particular gene on the DNA microarray or to the percentage of samples in a clonal complex (CC5, CC30, CC45) (**b**). Panels (**c**) and (**d**) present mined redescriptors for genotype to phenotype mapping of the patterns of *S. aureus* binding to granulocytes. The logical expressions above and below the thick lines are alternative redescriptors (solutions) that characterize the strains having the respective properties. Corresponding panels visualize intervals of functional values (MFI (**c**) and percentage of positive cells (**d**)) measured for the *S. aureus* isolates in a blood adhesion experiment at 5 min of co-incubating the bacteria and the whole blood together with the logical rules (queries) defining them in terms of the *S. aureus* genetic profile obtained from the DNA microarray hybridization profiles.

same run (granulocytes, monocytes, lymphocytes). We confirmed that *S. aureus* binding to professional phagocytes was time dependent with higher phagocytosis rates of granulocytes as compared to monocytes. Then, we provide the first evidence that *S. aureus* interacts with lymphocytes. Early but mostly transient binding was followed by persistent binding to a small subset of lymphocytes. Invasion of *S. aureus* into a subset of lymphocytes could be a potential immune escape mechanism resulting in *S. aureus* persistence and spreading *in vivo*. Using bioinformatics analysis, we demonstrated that *S. aureus* interaction with white blood cells can be associated with *S. aureus* clonal-complexes (genotypes) and also with methicillin resistance profiles (phenotypes). We suggest that the capacity of *S. aureus* binding and internalization is relevant for colonization, spreading and invasion also in the clinical setting.

3.2. Limitations of this work

One clear limitation is that all strains originated from nasal samples. This may introduce a certain bias of the results and their interpretation toward strains adapted to the nasal environment. A second limitation is that the isolates are representative only for those lineages predominating in one specific Southern German region. Although the number of isolates appears enough for a statistically sound comparison of the single feature MRSA yes/no, the findings of Fig. 3 are limited to the strains prevalent in South-West Germany that were investigated here. A third limitation is that for some clonal lineages, only 1–2 isolates were included, which would make it difficult to draw conclusions about clone-specific results. Realizing this, we have limited our clone-specific analysis (see Figs. 4 and 5) to those three clonal complexes that were represented by the largest numbers of isolates, CC5 (25 isolates), CC45 (13 isolates), CC30 (8 isolates). Hence, we believe that these results are fairly robust. Finally, it remains unclear to what extent these in vitro results are relevant in vivo for typical *S. aureus* infections. This cannot be answered based on the experiments presented here.

3.3. Findings of the present study in light of what was published before

Whether or not certain *S. aureus* clones are more virulent or pathogenic than others is unclear up to now (David, 2019). Whereas several previous studies were unable to connect invasive *S. aureus* diseases with specific phylogenetic groups (Feil et al., 2003; Lindsay and Holden, 2006; Rieg et al., 2013; Simor et al., 2016), an increased risk for haematogenous complications, such as endocarditis or septic arthritis, was shown for certain clonal complexes (CC5, CC30) (Fowler et al., 2007). Nienaber et al. (2011) showed that MSSA isolates related to infective endocarditis were significantly more likely to belong to CC30 and possess a distinct repertoire of virulence genes as compared to MSSA isolates causing soft tissue infections. Pérez-Montarelo et al. (2017) reported that 14 S. aureus strains representing CC5, CC8, CC15, CC30, and CC45 could all cause endovascular complications, but likely produce them through different mechanisms.

Our observation that CP5+ strains were engaged by granulocytes less strongly than CP8+ strains (Fig. 5 C) is in agreement with the work of Watts et al. (2005), who showed that CP5+ isogenic deletion mutants resisted *in vitro* killing in whole mouse blood and, once phagocytosed, survived intracellularly. CP5+ strains also showed higher virulence as compared to un-capsulated strains as well as to CP8+ mutants. In line with our findings, Clarke et al. (2007) could draw an association between the invasiveness of clinical *S. aureus* isolates and the presence of genes encoding *cap5*, adhesins such as *sasG* and *fnbB*, leukocidin genes *lukD/lukE*, as well as the genes encoding serine protease A and B (*splA/splB*), and the staphylococcal exotoxin-like protein (*setC* or *selX*). Iron-regulated surface protein A has already been associated with better survival in neutrophils and resistance to antimicrobial peptides. While we could not derive a direct association between the presence of enterotoxins genes (*egc*) with binding capacity, it was reported to be negatively correlated with the severity of infection and proposed to provide protection against severe sepsis (Ferry et al., 2005). Animal studies of *S. aureus* infection have demonstrated the importance of *cna* as a mediator of bacterial attachment to collagen-rich tissue (Rhem et al., 2000; Patti et al., 1994). However, by blocking the activation of the classical complement pathway, *cna* may also interfere with granulocyte recruitment and phagocytosis (Kang et al., 2013).

3.4. Understanding possible mechanism

Phagocytosis is the frontline defense against most bacteria, but *S. aureus* developed immune evasion strategies enabling persistence

within monocyte-derived macrophages for several days. Kubica et al. proposed that surviving phagocytosis and intracellular persistence of *S. aureus* does not affect the viability of infected monocyte derived macrophages, serving as a route of persistence and dissemination within the host (Kubica et al., 2008). Lymphocytes, as opposed to granulocytes to monocytes, have a longer life span increasing the probability of a shuttle function associated with unrecognized persistence and dissemination (Goenka et al., 2012). In accordance to the present results also other bacteria are able to invade lymphocytes, in particular B-cells, and to misuse invaded cells as a reservoir for reinfection and dissemination within a host (Souwer et al., 2012). Rosales-Reyes et al. (2012) showed that *Salmonella* take advantage of the unspecific micropinocytosis and also *Brucella abortus* was shown to find an intracellular niche in B-lymphocytes (Goenka et al., 2012). The difference of MRSA binding to granulocytes and monocytes could occur due to alternative binding receptors on granulocytes and monocytes; or due to competition between granulocytes and monocytes for planctonic bacteria in the whole blood. It would be interesting to study monocyte polarization by the different strains in future work.

3.5. Implications for practice or policy

With respect to a clinical relevance of delayed binding of MRSA we speculate that delayed phagocytosis could increase the probability of dissemination of unbound bacteria and the higher uptake of MRSA to monocytes offering intracellular shuttle functions could increase virulence and dissemination. The present functional testing of *S. aureus* binding to blood cells suggests that beside antibiotic resistance of MRSA also other strain dependent phenotypes as e.g. delayed interaction with blood cells may contribute to the high clinical impact and epidemic spreading of MRSA infections (Cosgrove et al., 2003).

4. Conclusions

Monitoring the interaction between *S. aureus* and blood cells emphasizes the importance of granulocytes and monocytes for phagocytosis of bacteria. For lymphocytes, we report for the first time a two-phase bacteria-cell-interaction. Phase 1 reflects early but preliminary binding of *S. aureus* to lymphocytes. Both, internalization into monocytes and lymphocytes may be associated with bacterial mimicry, persistence and spreading (shuttle) in vivo. Strain-dependent differences for *S. aureus* binding were related to CCs and antibiotic resistance types (MRSA vs. MSSA) and also to the presence of specific genes detected by DNA microarray. We present potential genetic markers correlating with particular behavioral phenotypic patterns for *S. aureus* binding to different white blood cells.

5. Materials and methods

5.1. Characterization of isolates

56 *S. aureus* isolates (Table S3) collected during an earlier hospital entry screening study of patients with nasal *S. aureus* colonization were included in the present study. Genotypes of these clinical isolates were comprehensively characterized via DNA microarray hybridization profiles (IdentiBAC, Alere, Jena, Germany), spa-typing and antibiotic susceptibility testing (Vitek 2, BioMerieux) as described earlier, see Fig. S1 of Ruffing et al., 2012. As this was previously done in a somehow condensed manner, we now provide the full processed microarray output in Table S3 of this manuscript. For completeness 29 of these isolates were methicillin-resistant (MRSA), 27 isolates were methicillin-susceptible (MSSA). The studied isolates were assigned to 8 different clonal complexes (CC5 (25 isolates), CC45 (13 isolates), CC30 (8 isolates), CC8 (2 isolates), CC97 (3 isolates), CC15 (2 isolates), CC398 (2 isolates) and CC22 (1 isolate)) and to 27 spa-types (t002, t003, t008, t010, t011, t012, t015, t019, t022, t026, t040, t045, t050, t073, t084, t267, t273, t481, t504, t571, t584, t620, t887, t1079, t1689, t2239, t8831).

5.2. Flow cytometric adhesion and internalization assay design

We employed a whole-blood flow cytometric assay which had also been used by us before for a phenotypic characterization of livestock-associated *S. aureus* isolates (Ballhausen et al., 2014). This assay with fluorescent-labeled clinical isolates enables to quantitatively analyze binding (adhesion and internalization) to the major cell types in human blood (granulocytes, monocytes, lymphocytes) as described earlier (Ballhausen et al., 2014). Please note that the assay does not discriminate between adhesion and internalization.

Isolates were cultivated overnight in brain heart infusion bouillon (BHI) (16 h, 37 °C, 150 rpm). The next day, 1.5 ml of the overnight cultures were washed twice with PBS (5 min, 1912 *g*). Optical density was measured using a spectrophotometer at 600 nm and a bacterial suspension with 1.5 OD_{600} was adjusted. After two additional washing steps, the bacterial pellet was re-suspended in 500 µl phosphate buffered saline (PBS) and fluorescent staining was performed by adding 2.5 µl 10 mM carboxy fluorescein diacetate succinimidyl ester (CFSE; Invitrogen) for 15 min at continuous shaking (1000 rpm). Fresh anticoagulated human whole blood (lithium heparin S-Monovette, Sarstedt) was taken from healthy volunteers, ranging from 20 to 45 years of age. 1000 µl of whole blood and 50 µl of the fluorescent- labeled bacterial suspension were co-incubated within 12- by 75-mm polystyrene tubes (Becton-Dickinson) at continuous shaking (8400 rpm). Samples of 200 µl were subsequently harvested at 1, 5, 15, 30 and 60 min of co-incubation. Then, erythrocytes were immediately lysed using 1 ml FACS lysing solution (Becton Dickinson) and supernatant was removed after centrifugation (450 rpm for 5 min). Finally, the cell pellet was re-suspended in FACS buffer (PBS with 2 % bovine serum albumin) and analyzed by flow cytometry using FACSCalibur (Becton Dickinson). The CellQuest Pro software was used to differentiate cells according to morphological properties (size and granularity) into distinct gates (cells types). In parallel, also the fluorescence intensity (FLI) was analyzed for fluorescently labeled bacterial binding. Thereby, quantitative analysis of the interaction between *S. aureus* with lymphocytes, monocytes, and granulocytes was available in the same run. The median fluorescence intensity and the percentage of positive cells were recorded. Detection of fluorescent blood cells was related in the present study to *S. aureus* binding without differentiation between adherence and internalisation.

5.3. Data analysis

5.3.1. Construction of a functional dendrogram

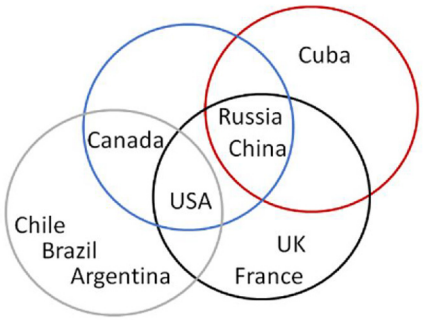
Clustering of *S. aureus* isolates was based on the mean fluorescent intensities and the fraction of positive cells. Dendrograms were constructed by Ward's hierarchical agglomerative clustering method. The statistical significance of the results was assessed with the Mann–Whitney *U* test All this was done with the “Stats” software package (R, version 2.13.1).

5.3.2. Redescription mining

Redescription mining (Kumar et al., 2004; Zaki and Ramakrishnan, 2005) was used to link available genotypic and phenotypic data with the software Siren (version 2.1.1 for Windows) using the ReReMi algorithm (Gallo et al., 2008; Galbrun and Miettinen, 2012). Based on the binary output of the DNA hybridization array (Ruffing et al., 2012) and the names of the *S. aureus* isolates, the algorithm generated pairs of queries (logical rules) that are fulfilled by a particular set of *S. aureus* isolates. Evaluation of the rules was based on the Jaccard coefficient (Jaccard, 1901), the number of entities covered ($|E_{1,1}|$), and the statistical p-value.

The task of redescription mining in our application is to effectively

A



B

Countries	Land area (km²)	History of communism	Popular tourist destinations in the Americas	Permanent members of the UN security council
Cuba	109820	1	0	0
Russia	**16377742**	1	0	1
China	**9326410**	1	0	1
UK	241930	0	0	1
France	549970	0	0	1
USA	**9147593**	0	1	1
Canada	**9093507**	0	1	0
Chile	743812	0	1	0
Brazil	8358140	0	1	0
Argentina	2736690	0	1	0

Fig. 6. Visualization of the exemplar data input to redescription mining. Colors of the overlapping circles (A) code for the objects (countries) sharing the description in one of the four features (B). In B "1" stands for "yes", "0" – "no".

describe the same subset of objects (e.g. bacterial isolates) by two vocabularies (genotypic and phenotypic assays readouts). Therefore we are looking for the clusters or subsets that are required to have not just one meaningful description, but two. The rationale behind the redescription mining is that the subsets that allow for such a two-way rule-based definition across two independent assessments are likely to exhibit a concerted behavior and are therefore of interest.

We continue with an example adapted from Parida and Ramakrishnan, 2005 to put the method into perspective. The input is a collection of objects flagged to belong to four groups by some meaningful definition. In this example colors blue, red, grey and black in Fig. 6 refer to the groups 'countries with land area > 9, 000, 000 square kilometers', 'countries with a history of communism', 'popular tourist destinations in the Americas' and 'permanent members of the UN security council'. We take these group assignments as examples for descriptions. An example of a redescription thereafter would be: 'Countries with land area > 9,000,000 square kilometers outside of the Americas' are the same as 'Permanent members of the UN security council who have a history of communism.' This redescription thereby defines the set {Russia, China} in two alternative ways, once by a set intersection, and once by a set difference.

5.4. Ethics statement

The study was performed in accordance to the declaration of Helsinki and the ethics committee (Chamber of Physicians of the State of Saarland, Ethics number 173/17). Written informed consent was obtained from all blood donors. *S. aureus* isolates were collected in a multicenter hospital admission study MRSAarNet (https://infectio-saar.de/) with written informed consent (Ethics number 127/10), samples were accordingly anonymized.

Author contributions

LVM designed this study and acquired BMBF funding for this project, AJ performed the experiments of this study and wrote the experimental methods section, DG performed data analysis and wrote the first draft of the manuscript, UR performed DNA microarray experiments, RA advised during data analysis, VH and LVM contributed to data analysis and discussions and edited the manuscript, MH contributed to study design and data discussion.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijmm.2020.151411.

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