



Population variation in anti-*S. aureus* IgG isotypes influences surface protein A mediated immune subversion



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ABSTRACT

Background: *Staphylococcus aureus* is a pathogen which causes life-threatening infection, the incidence of which rises during adult life. This, together with the emergence of drug-resistant strains and the expansion of more susceptible elderly populations, represents the rationale for the ongoing development of *S. aureus* vaccines targeting adult populations. Humoral responses to *S. aureus* naturally develop early in life, influence susceptibility to infection, and potentially influence the effect of vaccination. Despite this, the nature of pre-existing anti-*S. aureus* antibodies in healthy adult populations is not fully characterised. **Methods:** Immunoglobulin levels against *S. aureus* surface antigens were measured by a filter membrane enzyme-linked immunosorbent assay using fixed ΔSpA *S. aureus* as an antigen in serum samples obtained from three clinical cohorts comprising 133 healthy adult volunteers from 19 to 65 years of age. Functional capacity of antibody was also assessed, using antibody-mediated attachment of FITC-stained *S. aureus* to differentiated HL-60 cells.

Results: Wide variation in the concentrations of immunoglobulins recognising *S. aureus* surface antigens was observed among individuals in all three cohorts. There was a decline of anti-*S. aureus* IgG1 with age, and a similar trend was observed in IgM, but not in IgA or other IgG sub-classes. Antibody mediated bacterial attachment to cells was associated with IgG1 and IgG3 concentrations in serum. The presence of SpA on the bacterial cell surface reduced antibody-mediated binding of bacteria to phagocytes in serum with low, but not high, levels of naturally occurring anti-*S. aureus* IgG3 antibodies.

Conclusions: Naturally acquired immunoglobulin responses to *S. aureus* are heterogeneous in populations and their concentrations alter during adulthood. Elevated IgG1 or IgG3 titres against *S. aureus* enhance *S. aureus* recognition by phagocytosis and may be correlates of natural protection and/or vaccine efficacy in adult populations.

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1. Introduction

Staphylococcus aureus is a human pathogen primarily found in the anterior nares [1], and asymptomatic persistent carriage

of the bacterium occurs in ~30% of the general population [2]. *S. aureus* infections can range from mild skin conditions to invasive bacteraemia and pneumonia [3]. Persistent exposure to *S. aureus*, as occurs in *S. aureus* carriage, appears to confer some limited protection from some forms of *S. aureus* disease [4], while epidemiological data showing a gradual increase of the invasive disease incidence rate with increasing age, most marked from the age of about 40 years upwards [5,6], might be compatible with a slow decline of natural protection with ageing.

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S. aureus is listed by WHO as a growing healthcare and economic concern due to development of antibiotic resistance [7,8]. In the absence of new antibiotic discovery, vaccination is considered an alternative approach to control *S. aureus* infection. Humoral immunity mediates opsonisation of *S. aureus* and induces clearance by neutrophils [9,10]. Successful passive immunisation against the surface protein A (SpA) immune subversion antigen has been demonstrated in a murine challenge model [11]. Although *S. aureus* vaccines have, to date, failed in phase III clinical trials, new candidates inducing humoral immunity against cell surface proteins are in development [12–14]. Such vaccines will be deployed into a previously exposed human population, since antibody responses against *S. aureus* antigens start to rise in the first years of life and remain detectable through adulthood [15–17].

Immunoglobulin G (IgG) is the predominant isotype found in serum, comprising ~15% of plasma protein, and is further divided into 4 sub-classes (IgG1, IgG2, IgG3, and IgG4), classified in terms of their abundance and of functional differences [18,19]. While the Fab region of the antibody binds to specific antigen, the constant Fc region interacts with the host immune system, e.g. Fcγ receptors (FcγRs), to initiate downstream effects such as phagocytosis and antibody dependent cellular cytotoxicity, complement activation, and the release of reactive oxygen species [20–22]. Most clinical isolates of *S. aureus* express and secrete SpA [23], which sequesters human IgG sub-classes 1, 2 and 4 through high-affinity binding to the Fc region [24], thus interfering with antibody interaction with host cells and complement in *in vivo* and *in vitro*. As a result, *S. aureus* suppresses antibody-mediated immunity, impedes phagocytosis by human neutrophils, and interacts with B cell receptors inducing activation and subsequent cell death [25,26]. Thus, an effective antibody response against *S. aureus* has to overcome the immunomodulatory influence of SpA. Interestingly, its affinity for IgG3 is much lower and is allotype-specific [27], yet the levels of each IgG sub-class detecting *S. aureus* in individuals have not been investigated as being of relevance to SpA-mediated immune evasion.

Since naturally-acquired immunity against *S. aureus* is poorly understood, we investigated the variation of a range of isotypes of naturally-acquired antibodies against *S. aureus* within three healthy human populations, as well as their impact on SpA function. We discuss natural variation in titre and isotypes in the context of both vaccine response and natural protection from clinical infection in man.

2. Materials and methods

2.1. Experimental design and sampling

Antibody responses against *S. aureus* surface antigens were screened using serum collected from three separate cohorts of healthy adults, aged between 19 and 65 years old (Table 1).

Cohort A: Royal Navy submariners ($n = 50$) were involved in Surgeon General's Armed Forces Feeding Project, which included a study assessing the impact of *S. aureus* carriage on skin health (manuscript in progression), and provided written consent. A nasal swab and serum sample were taken between 24 and 72 h after consent was obtained, and before deployment. The medical officer on-board obtained a follow-up nasal swab from all submariners after a 40-day deployment. Serum samples were separated within 4 h of sampling and were stored initially at -20°C , then at -80°C . 14 out of 50 individuals were excluded from antibody analysis because of missing baseline questionnaire ($n = 6$), missing age data ($n = 1$), missing blood sampling ($n = 5$), missing post nasal swab ($n = 4$), and/or because of a technical problem with serum storage conditions ($n = 3$). 36 volunteer samples with complete clinical

Table 1
Demographic data of three clinical cohorts.

Cohort	A	B	C
Number of participants	36	26	71
Average age (years \pm S.E.)	31.9 \pm 1.34	35.7 \pm 1.45	38.1 \pm 1.45
Number of volunteers in each age group			
19–30	15	6	28
31–40	17	13	16
41–50	3	6	10
51–60	1	1	14
61–70	0	0	3
Male (rate)	36(1)	10(0.38)	25(0.35)
<i>S. aureus</i> nasal carriage (rate)	15(0.30)	8(0.31)	ND

ND, not determined.

data were analysed for serum antibody responses against *S. aureus* surface antigens.

Cohort B: Healthy adult volunteers, declaring themselves to be of Northern European ancestry, were recruited in Oxfordshire, UK ($n = 26$), as part of a *S. aureus* nasal carriage study (manuscript in progression). Exclusion criteria were: pregnancy, taking immunomodulatory drugs, diagnosis of cancer, connective tissue disease, blood borne viruses, or organ transplantation. Written consent was obtained, a questionnaire administered, and a nasal swab and blood taken on the day of recruitment. Serum samples were stored at -80°C . A second nasal swab was obtained between 1 and 2 months later from all subjects.

Cohort C: Healthy adult volunteers were recruited in Oxfordshire, UK ($n = 400$), for screening of healthy serum samples in immunoassays for vaccine development. There were no exclusion criteria for enrolment. Serum samples were stored at -80°C and 71 samples were randomly selected for antibody analysis.

2.2. Ethical approval

The study performed on Royal Navy servicemen (cohort A) was approved by the UK Ministry of Defence Research Ethics Committee (MODREC), Ref. 0903/228. The two human volunteer studies performed in Oxford (cohorts B and C) were approved by the National Research Ethics Service (NRES) Committee South Central (reference number 11/SC/0307), and NRES Committee South West (reference number 10/H0102/23), respectively.

2.3. Determination of nasal carriage

In both cohorts A and B, individuals with two positive swabs were considered persistent carriers [28]. Nasal samples were processed as described [28].

2.4. Enzyme linked immunosorbent assay (ELISA) for immunoglobulin isotyping

The assay was performed as described previously [29]. Briefly, 2×10^7 CFU/well paraformaldehyde (PFA)-fixed *S. aureus* spa::TcR isogenic DU5873 mutant [30] (obtained from Prof. Tim Foster, Trinity College, Dublin) (Δ SpA Newman strain) were immobilised on filter plates (Merck Millipore, MAGVS2210). Plates were blocked and incubated overnight with different serum concentrations. Plates were washed using MultiScreenHTS Vacuum Manifold (Merck Millipore) and incubated with various anti-human secondary antibodies [IgG1-HRP (Life Tech., MH1715); IgG2-ALP (Abcam, ab99783); IgG3-ALP (Abcam, ab99828); IgG4-ALP (Abcam, ab99822); IgG-ALP (Sigma, A3187); IgA-ALP (Sigma, A9669); IgM-ALP (Sigma, A3275)]. After further washing, the relevant substrate was added [tetramethylbenzidine or p-nitrophenyl phosphate

(Sigma)]. The reaction was stopped by addition of 2 N H_2SO_4 or 2 N NaOH (Scientific Laboratory Supplies), and 100 μL of the reaction was transferred into clean 96 well plates (NUNC). Absorbance was read at 450 nm or 405 nm, depending on substrate (CLARIOstar®, BMG Labtech). A 4 Parameter Logistic (4PL) nonlinear regression model was used for all ELISA assays for the interpolation from a reference serum. The concentration of each immunoglobulin isotype in this reference serum was assigned a concentration of 5 \log_{10} ELISA units per ml (\log_{10} unit/mL).

Specificity of binding for IgG and IgG1 sub-class was assessed by incubation of the target cells with IgG1 κ from human myeloma plasma (Sigma–Aldrich, I5154) at between 0 and 102 $\mu\text{g}/\text{mL}$, instead of serum. These experiments indicated that the DU5873 mutant did not bind detectably to the irrelevant IgG preparation.

2.5. Luciferase immunoprecipitation systems (LIPS) assay

The LIPS assay [31] was used to determine the IgG response against SpA_{KKAA}, a mutated SpA D domain lacking Fc binding activity [32]. Briefly, plasmid constructs of the SpA_{KKAA} sequence fused with renilla luciferase were transfected into HEK293 cells and cell lysates stored at -80°C for further use. Serially diluted human serum was incubated with cell lysate, then added to Protein G UltraLink Resin (ThermoScientific) in filter plates (Merck Millipore, MSGVN2B50). After overnight incubation and subsequent washing, assay substrate was added (Renilla luciferase assay system, Promega UK Ltd) and chemoluminescence measured in a Lumimeter (CLARIOstar®, BMG Labtech). A 4PL nonlinear regression model was used for the interpolation from a reference serum. The concentration of this reference serum was assigned a concentration of 4 \log_{10} luciferase activity units per ml (\log_{10} luciferase unit/mL).

2.6. Antibody-mediated interaction of differentiated HL-60 cells with *S. aureus*

29 serum samples from cohorts A and B were randomly selected, heat inactivated at 56°C for 30 min, then stored at -80°C until used. The acute myeloid leukaemia cell line HL-60 cells (obtained from Prof. Andrew Pollard) were cultured in RPMI 1640 medium containing 2 mM L-glutamine supplemented with 10% foetal bovine serum (all from Sigma) with 100 mM N,N-dimethylformamide (Fisher Scientific) for 3 days for differentiation to Fc γ R expressing phagocytes [33]. *S. aureus* (wild-type and ΔSpA Newman strains) were incubated in 10 ml tryptic soya broth (Oxoid) overnight and used to set fresh cultures for 1.5 h growth. Bacteria were washed and stained with FITC (Sigma), then incubated with heat-inactivated serum for 10 min at 37°C with shaking at 130 rpm. Antibody-coated bacteria were washed and resuspended in medium. Differentiated HL-60 cells in medium were added to the wells containing bacteria (2×10^5 cells to 1×10^6 CFU per well) and incubated at 37°C for 15 min with shaking at 130 rpm. Samples were placed on ice immediately after incubation and cells were stained with Aqua live/dead stain (Invitrogen) then fixed with 4% PFA. Data was acquired with a CyAn7 cytometer and analysed with Summit analysis software V4.3.01 (Dako Colorado, Inc.). Antibody-mediated binding of bacteria to phagocytes was measured as a percentage of cell-associated fluorescence (FITC⁺ HL-60 cells) (Fig. 3A–F), where the background signal was $2.76 \pm 0.60\%$ without pre-incubation of bacteria with serum (Fig. 3E). Inhibitory effect of SpA on antibody-mediated binding of bacteria to phagocytes per sample was calculated by: (% HL-60 cells with FITC⁺ ΔSpA) – (% HL-60 cells with FITC⁺ wild-type).

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.03, IBM SPSS Statistics version 22, and R 3.1.1 for Windows. Analysis was performed on \log_{10} transformed data, using linear regression and generalised linear modelling with Wald-chi square test, and Spearman rank correlation tests. Linear modelling of interaction assay was performed using the glm function in R.

3. Results

3.1. IgG1 levels against *S. aureus* surface antigens show decline with age

Serum IgM, IgG and IgA levels were measured against ΔSpA *S. aureus* Newman strain in cohort A. There was a wide variation in baseline levels of these immunoglobulins recognising the *S. aureus* surface (Fig. 1), with about 10-fold variation between individuals. There were trends towards decline of IgM ($p = 0.052$) and IgG binding ($p = 0.08$) to *S. aureus* surface antigens with increasing age (Fig. 1A and B), while anti-*S. aureus* IgA levels were more stable (Fig. 1C). Of all the IgG sub-classes, only anti-*S. aureus* IgG1 levels showed a negative correlation with age (Fig. 1E–H). Since the antigen lacked SpA, anti-*S. aureus* IgG response against a recombinant SpA domain with KKAA mutations [32] which abrogate Fc binding, was also measured. The anti-SpA_{KKAA} IgG response was not significantly associated with age (Fig. 1D), so it is possible that age-specific decline in IgG titres is restricted to only some cell surface antigens.

To determine whether the pattern seen in the submariner cohort, all of which was male, was representative of the general population, serum IgG1 levels against ΔSpA *S. aureus* surface antigens were analysed in healthy adults in Oxfordshire (cohort B and C). The inverse relationship of anti-*S. aureus* IgG1 with age was confirmed in these populations (Fig. 2A), and this was evident in both genders (Fig. 2B). *S. aureus* nasal carriage status was determined in cohort A and B (Table 1), and an age-associated anti-*S. aureus* IgG1 decline was observed in both *S. aureus* nasal carriers and non-carriers (Fig. 2C). Notably, and similarly to the result observed with cohort A, the levels of anti-*S. aureus* IgG3 did not decline significantly with age in either cohorts B or C (Fig. 2D). Of note, most of the subjects studied (120/136) were aged between 19 and 50, and the age associated decline observed was driven by these individuals (Spearman's $\rho = -0.2874$ $p = 0.002$, for those age 50 or under).

3.2. IgG levels against ΔSpA *S. aureus* surface antigens correlates with functional capability

Uptake of antibody-opsonized pathogens by phagocytes, through Fc-mediated receptor binding [34], is an important function of pathogen specific immunity. To assess this, we adapted the opsonophagocytic assay using the HL-60 cell line [33], which can be differentiated into Fc receptor positive neutrophil-like cells *in vitro*, providing a consistent supply of phagocytic cells for assessing Fc-receptor mediated pathogen binding, and fluorescent labelled bacteria to measure the rate of bacteria-bound cells by flow cytometry [35,36]. *In vitro* culture of opsonised FITC⁺ *S. aureus* ΔSpA with HL-60 cells showed a positive correlation between serum anti-*S. aureus* IgG titres and percentages of cell-associated fluorescence, suggesting that anti-*S. aureus* IgG levels correlate with functional capacity to mediate phagocyte–antigen interaction (Fig. 3G).

If this cell-associated fluorescence requires Fc binding of antibody to Fc receptors, the interaction should be reduced by SpA, which mediates non-specific immunoglobulin Fc binding [24]. The same assay was performed using wild-type *S. aureus*; cell-associated fluorescence was reduced in presence of SpA for most

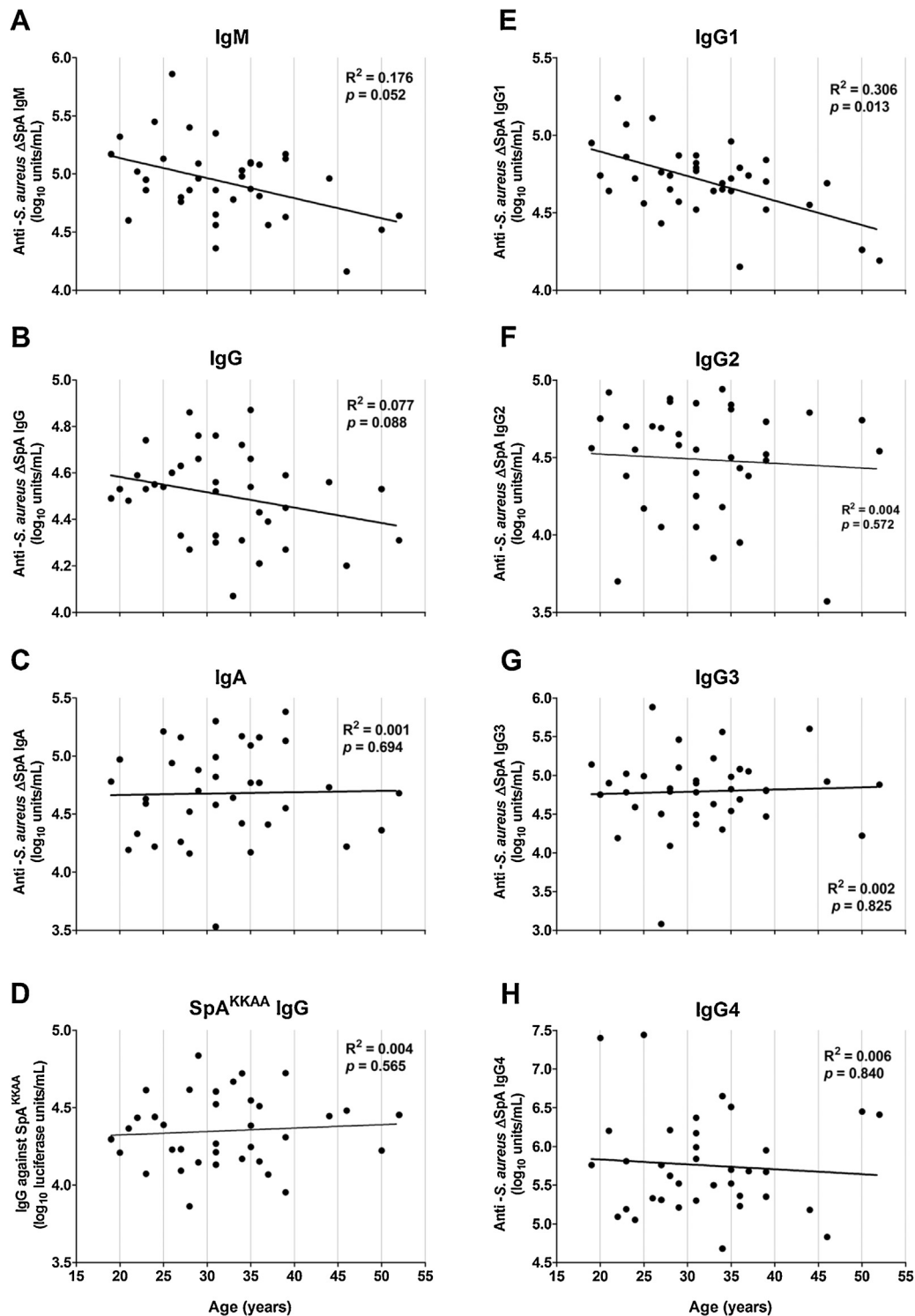


Fig. 1. Association of age and immunoglobulin levels against *S. aureus* in cohort A. Levels of IgM (A), IgG (B) and IgA (C) binding to *S. aureus* ΔSpA surface antigens, luciferase activity representing IgG titre against *S. aureus* SpA^{KKAA} D domain (D), levels of IgG sub-classes binding to *S. aureus* ΔSpA surface antigens (E–H): all plotted against age.

samples, however the positive correlation persisted between anti-*S. aureus* IgG titre and cell-associated fluorescence (Fig. 3H). However, the inhibitory effect of SpA, measured as the difference between the cell-associated fluorescence of ΔSpA and wild-type *S. aureus*, diminished as anti-*S. aureus* IgG titres increased (Fig. 3I). Thus, endogenous levels of IgG against *S. aureus* surface antigens correlates with functional activity, and the immunoevasive effect of SpA is greater at lower concentrations of anti-*S. aureus* IgG.

3.3. High serum IgG3 titre against *S. aureus* diminishes the immunoevasive effect of SpA

Affinities of SpA to each IgG sub-class are different, thus negative association of anti-*S. aureus* IgG titre and inhibitory effect of SpA may be differently influenced by the concentration of each IgG sub-class. Correlations between percentages of cell-associated fluorescence with wild-type *S. aureus* and levels of anti-*S. aureus* IgG

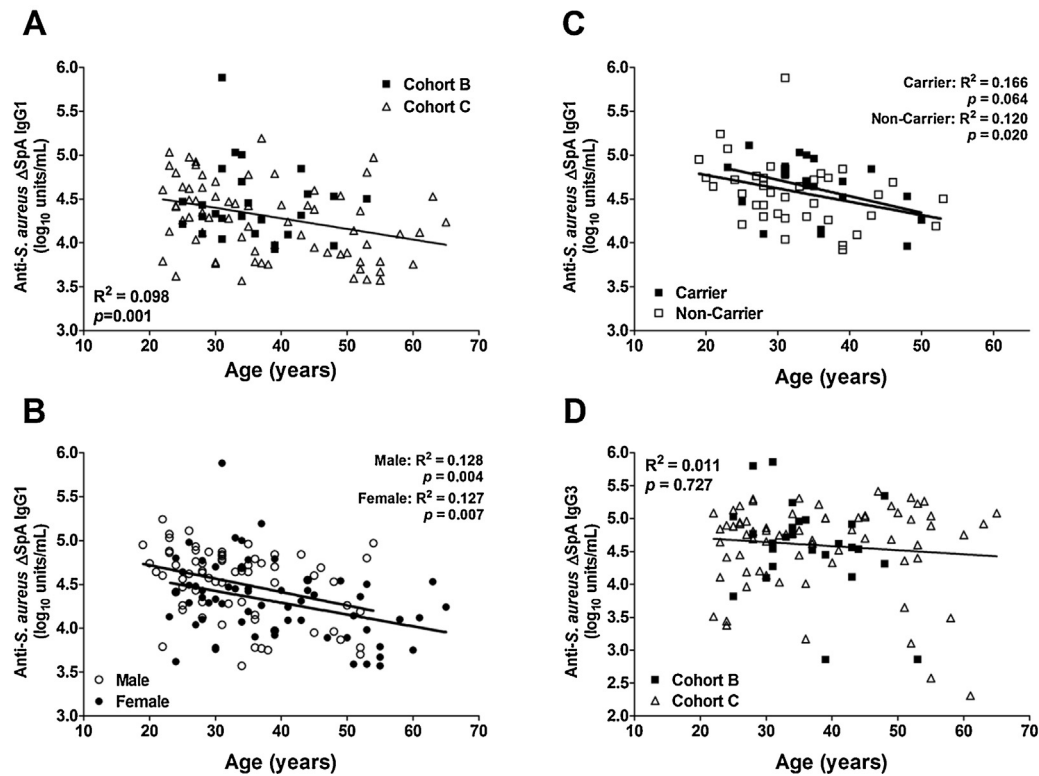


Fig. 2. Association of IgG1 and IgG3 levels against *S. aureus* to age, gender and *S. aureus* carriage status. (A) Correlation plots of IgG1 responses to *S. aureus* ΔSpA surface antigens vs. age in cohort B (closed squares) and cohort C (open triangles). Comparison of age associated anti-*S. aureus* IgG1 decline between gender (B: cohort A–C) and *S. aureus* nasal carriage status (C: cohort A and B). (D) Correlation plots of IgG3 response to *S. aureus* ΔSpA surface antigens vs. age (cohort B and C).

sub-classes showed a strong positive relationship between levels of anti-*S. aureus* IgG3 and cell-associated fluorescence of wild-type *S. aureus*, while there were no associations with IgG1, IgG2 or IgG4 using univariate analysis (Fig. 4, top panels). Anti-*S. aureus* IgG sub-class titres within the general population displayed only weak correlations with each other (maximum $r = 0.27$ between sub-classes, Table 2), which allowed multivariate analysis, modelling cell-associated fluorescence of wild-type *S. aureus* as a function of IgG1, 2, 3 and 4 concentrations. A multivariate model supported a contribution of IgG3 to cellular-bacterial interaction, independent of other sub-classes ($p = 2.2 \times 10^{-6}$ in multivariate model). It also suggested that an independent role may be played by anti-*S. aureus* IgG1 ($p = 0.007$ in multivariate model).

Levels of anti-*S. aureus* IgG sub-classes were then compared with the impact of SpA on cell-associated fluorescence. There was a negative correlation between effect of SpA and anti-*S. aureus* IgG3 titres, but not with titres of other IgG isotypes (Fig. 4, bottom panel). Thus antibody-mediated binding of cells and *S. aureus* was influenced by concentrations of anti-*S. aureus* IgG3 and IgG1 in serum, and that increased concentrations of IgG3 within the physiological range can overcome the effect of SpA.

Table 2
Correlation matrix of IgG sub-classes. Pearson correlation coefficients for $n = 29$ individuals of cohort A and B, the sera of which was used to assess antibody-mediated binding of HL-60 cells and FITC⁺ wild-type *S. aureus*.

	IgG1	IgG2	IgG3	IgG4
IgG1	1.00	−0.20	−0.09	−0.25
IgG2	−0.20	1.00	0.24	0.27
IgG3	−0.09	0.24	1.00	−0.23
IgG4	−0.25	0.27	−0.23	1.00

4. Discussion

This paper describes an age-associated decline of naturally induced IgG1 antibodies against *S. aureus* surface antigens, and heterogeneity of concentrations of immunoglobulin isotypes against *S. aureus* in healthy adults between 19 and 65 years old. This age-dependent decline in IgG1 titre against *S. aureus* surface antigens is congruent with reports of a decline in IgG responses to ClfB, a *S. aureus* surface antigen, with increasing age [17]. While naturally acquired human IgG1 and IgG3 titres against *S. aureus* were associated with antibody-mediated *S. aureus* attachment to phagocytes, attachment was attenuated by SpA in sera with low, but not high, IgG3 levels. Since antibody-dependent uptake and killing of *S. aureus* by neutrophils is the primary mechanism of bacterial clearance by the host immune system [10,37], the naturally occurring variation in antibody responses, attenuated by SpA to different degrees between individuals, may influence both natural protection from infection and post-vaccination immune response in man. Since IgM and IgG1 are the major immunoglobulin isotypes present in serum, we suggest that the age-associated decline observed in this study is likely to contribute to an increased risk of invasive *S. aureus* disease in the middle-aged to elderly [38]. The implication is that boosting of immune responses in the middle-aged population, before the waning of adaptive immunity to sub-optimal levels [39,40] and prior to the period of highest risk, as occurs with immunity to *Varicella zoster virus* [41], may improve later-life immune responses. Of note, the study cohorts were mainly composed of individuals from 19 to 50 years old, with none over 65 years old. The effect described was observed if one restricts to individuals under 50 years of age. Therefore, one could speculate that a further decline of IgG1, and possibly IgM, against *S. aureus* would be observed in the population above 65 years of age. This, together with an exploration of whether such effects also occur against

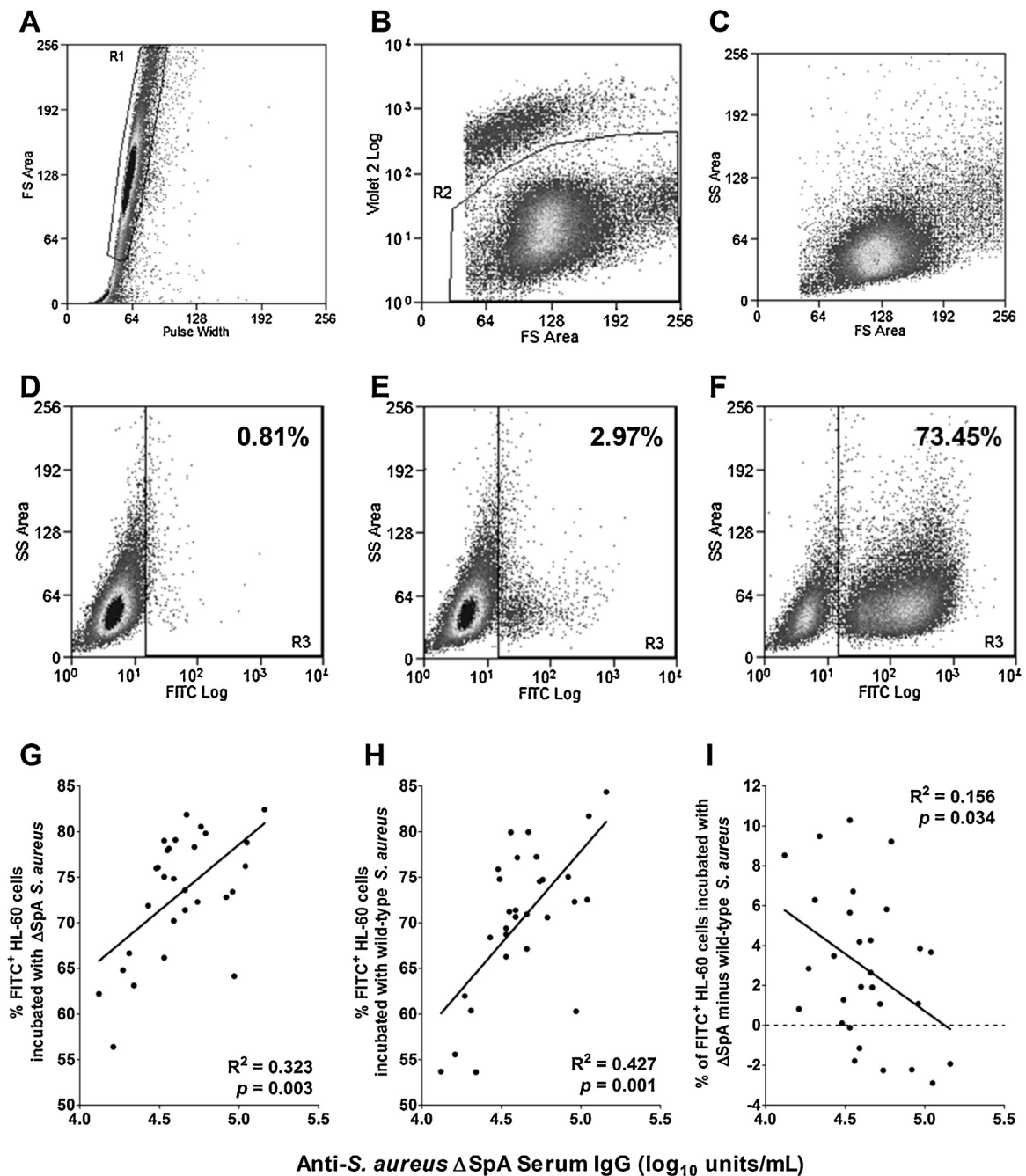


Fig. 3. Detection of antibody-mediated binding of FITC⁺ *S. aureus* to differentiated HL-60 cells. (A–F) FACS analysis gating strategies; cells were gated for singlets (A) then AQUA negative live cells (B) before analysing percentage of FITC⁺ cells. Live cells were plotted on FCS vs SSC (C). Example plots of differentiated HL-60 cells alone (D), differentiated HL-60 cells with FITC⁺ *S. aureus* without serum pre-treatment (E), and differentiated HL-60 cells with FITC⁺ *S. aureus* pre-treated with heat-inactivated serum (F) with percentages of FITC⁺ HL-60 cells in each gate. (G) Percentage of differentiated HL-60 cells attached to FITC⁺ ΔSpA *S. aureus*, (H) percentage of differentiated HL-60 cells associated with FITC⁺ wild-type *S. aureus*, (I) percentage difference of differentiated HL-60 cells associated with FITC⁺ ΔSpA *S. aureus* minus FITC⁺ wild-type *S. aureus*; all plotted against ELISA IgG response to ΔSpA *S. aureus* surface antigens.

other bacterial pathogens, including Gram negative pathogens, is an important area for further investigation. In this study, although modelling indicates that IgG1 (which declines during adult life) contributes to opsonophagocytosis, it was notable that the anti-*S. aureus* IgG3 response was found to be independent to the effect of ageing, and the concentrations of this sub-class correlated to the interaction of phagocytes with wild-type *S. aureus*, presumably due to its low affinity for Fc-mediated sequestration by SpA. IgG3 dependent protection may also operate by other means, such as specific enhancement of antibody dependent cellular cytotoxicity, as suggested by analysis of the ALVAC-HIV ('Thai trial') in which

IgG3 levels against HIV were associated with protection [42]. Stability of anti-*S. aureus* IgG3 responses across the age groups indicates that naturally acquired levels of anti-*S. aureus* IgG3 alone cannot maintain the level of protection required. Considering its low abundance and short half-life in serum [19], induction of IgG3 responses along with the maintenance of IgG1 levels may be possible correlate of vaccine efficacy and natural protection in the population above middle age.

One limitation concerns the use of serum as a source of antibodies during our *in vitro* studies. Physiologically the site of interaction of antibody with the cell surface of *S. aureus* is usually mucosal, and

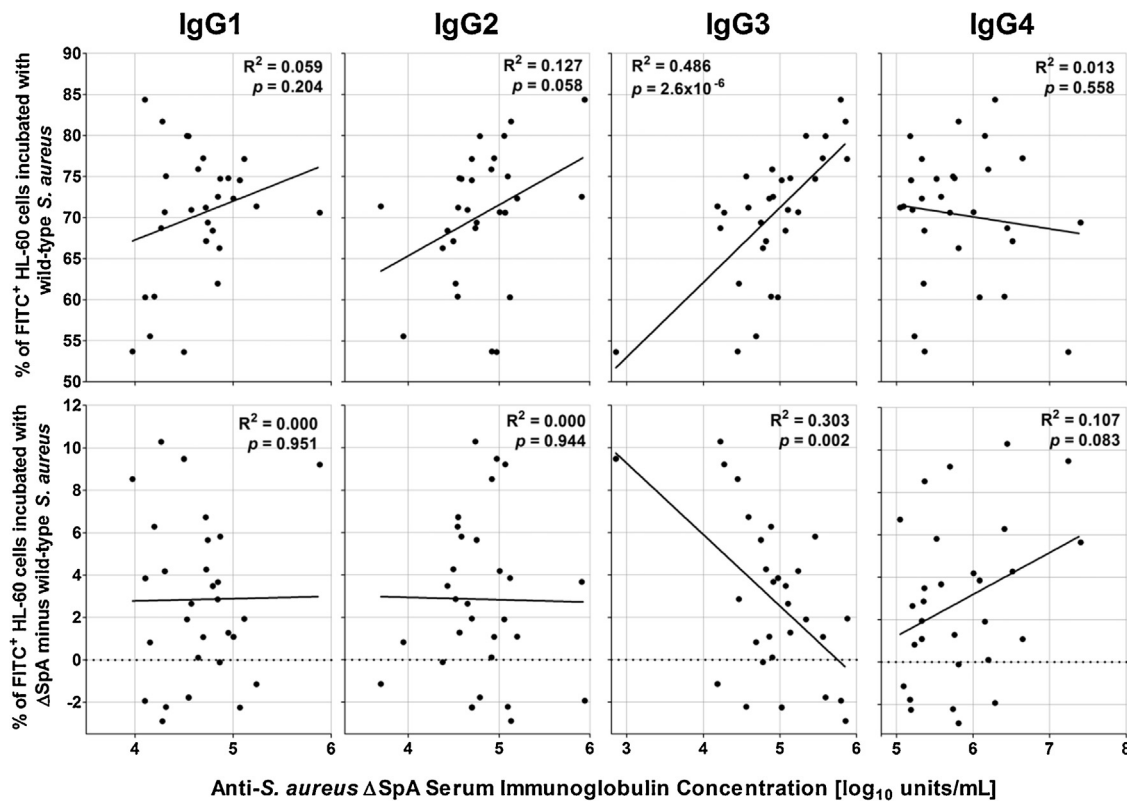


Fig. 4. Association of % FITC⁺ HL-60 cells and anti-*S. aureus* IgG sub-class levels. Percentage of differentiated HL-60 cells associated with FITC⁺ wild-type *S. aureus* (top panels), and percentage difference of cell-associated fluorescence of Δ SpA and wild-type *S. aureus* (bottom panels), plotted against serum IgG1–4 levels specific to Δ SpA *S. aureus* surface antigens. p values shown on each plot refer to hypothesis of no association between IgG concentrations and fluorescence, and was derived from univariate generalised linear modelling.

functional antibody–bacterium interactions at this site have been studied for *Streptococcus pyogenes*, another IgG binding bacterium [43]. Antibody concentrations in mucosal fluids, such as saliva, are much lower than in serum, and the impact of SpA on the function of the sub-classes present in the saliva of human populations may be much more pronounced than that evident in our studies using serum, as described for *S. pyogenes* [43]. This area could be studied further.

Independent of the mechanisms behind IgG sub-class action against *S. aureus*, the data presented suggest emphasis needs to be placed on the quality of the immune response generated both by natural exposure and vaccination programmes. In particular, we suggest that the age-associated decline of anti-*S. aureus* immunoglobulin isotypes, particularly IgG1, perhaps combined with the documented decline in neutrophil function, including phagocytosis and respiratory burst, which occurs with age [44], may contribute to rising *S. aureus* susceptibility in the vulnerable patient group. Better understanding of naturally-acquired immunity, correlated with epidemiological data may assist logical vaccine design when attempting to protect a population with heterogeneous pre-existing immunity against *S. aureus* infection.

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