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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.

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

Table 1
Demographic data of three clinical cohorts.

Cohort	A	B	C
Number of participants	36	26	71
Average age (years ± S.E.)	31.9 ± 1.34	35.7 ± 1.45	38.1 ± 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)
S. aureus nasal carriage (rate)	15 (0.30)	8 (0.31)	ND

ND, not determined.

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) (ΔSpA 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