ORIGINAL ARTICLE

Induction of antibodies by *Staphylococcus aureus* nasal colonization in young children

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Abstract

In order to develop novel antistaphylococcal strategies, understanding the determinants of carriage and how humans respond to *Staphylococcus aureus* exposure is essential. Here, the primary *S. aureus*-specific humoral immune response and its association with nasal colonization was studied in young children. Sera from 57 colonized or non-colonized children, serially collected at birth and at 6, 14 and 24 months, were analysed for IgG, IgA and IgM binding to 19 staphylococcal proteins, using flow cytometry-based technology. The antibody responses showed extensive inter-individual variability. On average, the levels of antistaphylococcal IgA and IgM increased from birth until the age of 2 years ($p < 0.05$), whereas the levels of IgG decreased ($p < 0.001$). Placentally transferred maternal IgG did not protect against colonization. In colonized children, IgG and IgA levels for a number of proteins were higher than in non-colonized children. At both 14 and 24 months, the levels of IgG against chemotaxis inhibitory protein of *S. aureus* (at 24 months; median fluorescence intensity, 4928 vs. 24, $p < 0.05$), extracellular fibrinogen-binding protein (987 vs. 604, $p < 0.05$), and iron-responsive surface determinant H (62 vs. 5, $p < 0.05$) were significantly higher in colonized children. The levels of IgA against CHIPS, IsdH and IsdA were higher ($p < 0.05$). Therefore, CHIPS, Efb, IsdA and IsdH seem to play a role in nasal colonization of young children.

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Introduction

Staphylococcus aureus efficiently colonizes human skin and, most frequently, nasal mucosa [1]. Approximately 20–30% of adults carry *S. aureus* persistently, and approximately 70–80% of adults carry *S. aureus* never or intermittently [2,3]. *S. aureus* is carried by 10–35% of children [4]. During the first 2 months of life, the prevalence of colonization is 40–50%. Then, the prevalence rapidly decreases to approximately 20% by 6 months and to 10% by 14 months [5,6]. How nasal carriage is established and maintained is still largely unknown [2,7], although the involvement of bacterial components such as teichoic acid, catalase, hydroperoxide reductase, iron-responsive surface determinant A (IsdA), *S. aureus* surface protein G and clumping factor B (ClfB) has been demonstrated [8–13].

Carriage of *S. aureus* can result in serious endogenous infections. Because of the increasing antibiotic resistance of *S. aureus*, novel approaches concerning the prevention and therapy of staphylococcal disease are urgently needed. In order to develop such new strategies, understanding the determinants of carriage and understanding how humans respond to *S. aureus* exposure is essential. Here, we provide insights into the antistaphylococcal humoral immune response in young children. Studying their immune response will allow us to distinguish the bacterial factors that are expressed *in vivo* during early colonization. This may lead to the discovery of novel determinants of colonization.

Materials and Methods

Study population

This project was performed with a subgroup of the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards [14,15]. The infants were presented at the Generation R

research centre at the ages of 1.5 months, 6 months, 14 months and 24 months. Research nurses obtained a nasal swab for *S. aureus* isolation from each infant at each visit, whenever possible. The methods of nasal sampling and identification of *S. aureus* were as described previously [5]. Serum samples were collected from cord blood and through venipuncture at 6 months, 14 months and 24 months whenever possible. Included in this study were 57 healthy children, from each of whom three or four serial serum samples were collected. Of the 177 samples that were obtained, 54 (31%) were cord blood samples; 32 samples (18%) were obtained at 6 months, 46 (26%) at 14 months, and 45 (25%) at 24 months. *S. aureus* colonization data were available at 1.5, 6, 14 and 24 months for 40 (70%), 49 (86%), 50 (88%) and 48 (84%) children, respectively. Children were classified as colonized if at least one of the nasal swab cultures was positive for *S. aureus*. Children were classified as non-colonized if all swab cultures were negative. Children with a culture moment missing at one time-point, and with the other nasal swab cultures negative, were classified as non-colonized as well.

None of the children suffered from apparent staphylococcal infection. The Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands, approved the study. Written informed consent was obtained from the parents of all participating children.

Antistaphylococcal antibodies

The levels of antistaphylococcal antibodies directed against three important groups of *S. aureus* proteins, 'microbial surface components recognizing adhesive matrix molecules' (MSCRAMMs), staphylococcal enterotoxins (SEs) and immunomodulatory proteins, were determined. The proteins have been described previously [16]. MSCRAMMs are generally considered to be important for host colonization [12,17]. The recombinant MSCRAMMs clumping factor A (ClfA), ClfB, *S. aureus* surface protein G, IsdA, iron-responsive surface determinant H (IsdH), fibronectin-binding protein A, fibronectin-binding protein B, serine–aspartate dipeptide repeat protein D (SdrD) and serine–aspartate dipeptide repeat protein E were used. SEs are superantigens, and therefore potent pro-inflammatory agents [18]. The recombinant proteins SEA, SEB, SEI, SEM, SEO, SEQ and toxic shock syndrome toxin-1 were used. Recombinant staphylococcal complement inhibitor (SCIN), extracellular fibrinogen-binding protein (Efb) and chemotaxis inhibitory protein of *S. aureus* (CHIPS) were also used. Efb and SCIN are complement inhibitors that lead to a reduction of bacterial phagocytosis and killing by human neutrophils [19,20]. CHIPS impairs the response of neutrophils and monocytes to formylated peptides and complement factor C5a [21].

The levels of antigen-specific IgG, IgA and IgM were quantified using the bead-based flow cytometry technique (xMap; Luminex Corporation, Austin, TX, USA). The methods used were as described previously [16,22,23]. Tests were performed as independent duplicates, and the median fluorescence intensity (MFI) values, reflecting antibody levels semiquantitatively, were averaged. In each experiment, control beads (no protein coupled) were included to determine non-specific antibody binding. In cases of non-specific binding, the median fluorescence intensity values were subtracted from the antigen-specific values. Human pooled serum was used as a standard.

Statistical analysis

Statistical analyses were performed with SPSS version 15.0. The Wilcoxon signed rank test was used to compare the antistaphylococcal antibody levels between different age groups. Mann–Whitney *U*-tests were used to compare differences in antibody levels between colonized and non-colonized children. Binary logistic regression analysis was used to determine the relationship between maternal IgG levels and the dichotomous outcome colonization. A p-value ≤ 0.05 was considered to be statistically significant.

Results

Dynamics of the antistaphylococcal antibody response

The changes in antistaphylococcal IgG, IgA and IgM levels during the first 2 years of life were determined (Fig. 1; data shown for toxic shock syndrome toxin-1, ClfB and Efb). The levels of antigen-specific IgG, IgA and IgM showed extensive inter-individual variability over time. For all *S. aureus* proteins tested, the level of antigen-specific IgG in cord blood was significantly higher than the antistaphylococcal IgG level at 6 months ($p < 0.001$). This was due to the presence of maternal IgG at birth and catabolism of maternal IgG thereafter. In the time interval from 6 to 14 months, the levels of IgG directed against CHIPS, SCIN and SEB decreased further ($p < 0.05$). As for the other proteins, no significant change in IgG levels was noted in this period.

Antistaphylococcal IgA and IgM levels in cord blood were low, because maternal IgA and IgM are not transported across the placenta. In the first 2 years of life, IgA levels remained low, which is a well-known fact [24,25]. However, for both IgA and IgM, a significant increase from birth up to the age of 24 months was noted, for 18 of 19 *S. aureus*

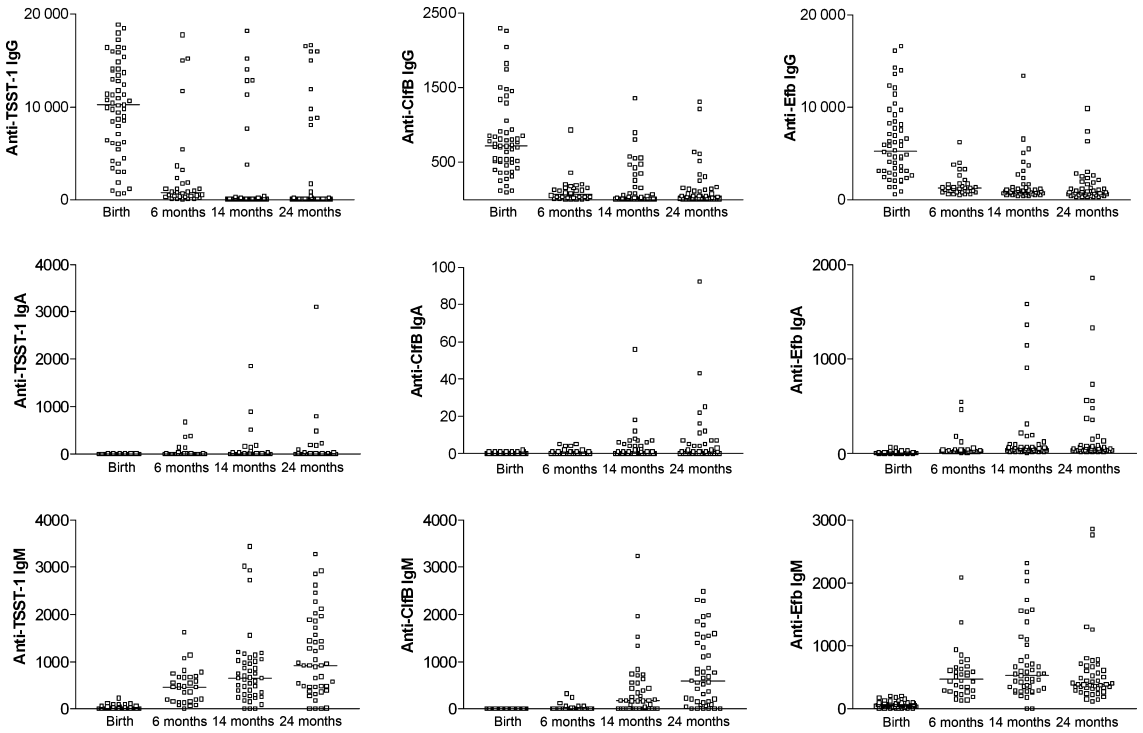

FIG. 1. Levels of IgG, IgA and IgM directed against toxic shock syndrome toxin-1 (TSST-1), clumping factor B (ClfB) and extracellular fibrinogen-binding protein (Efb) in 57 children at birth, 6 months, 14 months and 24 months. Antibody levels are reflected by median fluorescence intensity values. Each dot represents a serum sample. Median values are indicated by horizontal lines.

proteins in the case of IgA ($p < 0.05$, with the exception of anti-SCIN IgA) and for all proteins in the case of IgM ($p < 0.01$). It must be emphasized that not every infant developed an antigen-specific IgA or IgM response to each protein in the first 2 years of life. Within one individual, the level of IgG, IgA or IgM directed against one protein was not correlated with the level of IgG, IgA or IgM directed against another protein.

Relationship between colonization and antistaphylococcal antibody levels

It was determined whether maternal antistaphylococcal IgG levels were predictive of the *S. aureus* colonization state of the infant and whether the colonization state determines the level of antistaphylococcal antibodies. Levels of *S. aureus*-specific IgG in cord blood were not predictive of the colonization state at 1.5 and 6 months ($p > 0.05$). This implies that the large amounts of placentally transferred maternal IgG do not protect children from becoming nasally colonized with *S. aureus*.

For 45 of 46 (98%) children from whom serum samples were obtained at 14 months, the colonization status was known. For one child, the colonization status could not be determined, because two nasal swab cultures were missing. In the first year of life, 24 (53%) children were colonized at least once, and 21 children (47%) were not colonized. Colonized children had significantly higher levels of IgG directed against CHIPS, Efb, ClfB, SdrD and IsdH than non-colonized children ($p < 0.05$; Fig. 2). In addition, their levels of IgA directed against CHIPS, IsdA and IsdH were higher ($p < 0.05$). Incidentally, high levels of antibodies were detectable in non-colonized children, probably owing to exposure to *S. aureus* that was not recorded during this study. For 42 of 45 (93%) children from whom serum samples were obtained at 24 months, the colonization status was known. In the first 2 years of life, 24 (57%) children were colonized at least once, and 18 (43%) were not colonized. Colonized children had higher levels of IgG directed against CHIPS, SCIN, Efb, IsdA, IsdH and SEB at 24 months than non-colonized children ($p < 0.05$; Fig. 2). Their levels of IgA directed against CHIPS, IsdA and IsdH at 24 months were higher as well ($p < 0.05$). The levels of IgM did not differ significantly between colonized and non-colonized children ($p > 0.05$).

Discussion

Understanding the determinants of carriage and how humans respond to *S. aureus* exposure is important for the development of novel antistaphylococcal measures. We show that,

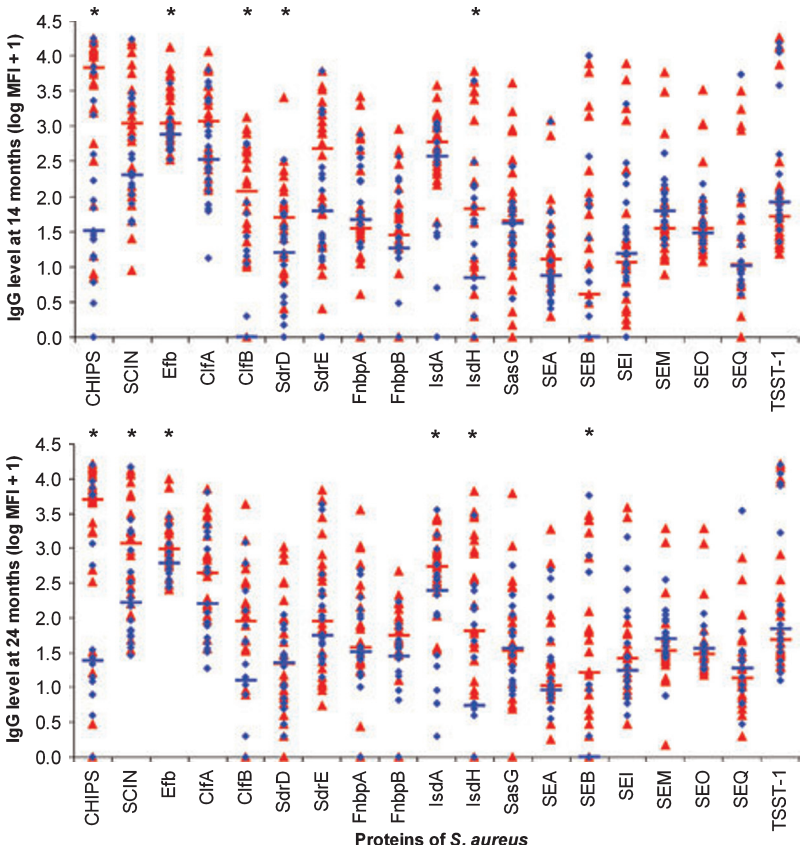

FIG. 2. (a) Relationship between *Staphylococccus aureus* colonization in the first year of life and levels of antistaphylococcal IgG, reflected by median fluorescence intensity (MFI) values, at 14 months. Each symbol represents a single child. Red triangles represent colonized children, and blue diamonds represent non-colonized children. Median values are indicated by horizontal lines. (b) Relationship between *S. aureus* colonization in the first 2 years of life and the level of IgG at 24 months. CHIPS, chemotaxis inhibitory protein of *S. aureus*; SCIN, staphylococcal complement inhibitor; Efb, extracellular fibrinogen-binding protein; Clf, clumping factor; Sdr, serine–aspartate dipeptide repeat protein; Fnbp, fibronectin-binding protein; Isd, iron-responsive surface determinant; SasG, *S. aureus* surface protein G; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin-1.

despite extensive inter-individual variability, the levels of IgG and IgA directed against a number of *S. aureus* proteins were significantly higher in colonized, more exposed children than in non-colonized children. In both the first and second years of life, anti-CHIPS, anti-Efb and anti-IsdH IgG levels were higher in colonized children. Furthermore, anti-CHIPS, anti-IsdA and anti-IsdH IgA levels were higher. This indicates that these proteins are expressed *in vivo* and that they might be determinants for colonization in early childhood. A potential role of IsdA in colonization was demonstrated in a previous study [10].

Furthermore, we show that maternally derived IgG antibodies specifically directed against a series of staphylococcal antigens do not seem to protect the infant against *S. aureus* nasal colonization in the first months of life. Thus, although it is known that maternal IgG can cross epithelial barriers and reach significant levels at the nasal mucosal surface [26,27], these antibodies are not capable of preventing nasal colonization. In healthy adults, the considerable levels of antistaphylococcal antibodies that are found do not seem to protect against nasal colonization either. Carriers have even higher levels of antibodies than non-carriers [16]. These observations suggest that attempts to prevent mucosal colonization by *S. aureus* through passive immunization approaches are not likely to succeed. Whether this also applies to active immunization remains to be elucidated.

The present study was limited by the fact that the *S. aureus* nasal swabs and serum samples were not available at all times for all children. Furthermore, our study focused exclusively on antibodies directed against *S. aureus* proteins. It should be noted that cell wall components such as capsular polysaccharides 5 and 8 [28], peptidoglycan [29] and teichoic acid [8] are immunogenic as well.

In summary, in healthy children, the antistaphylococcal IgG, IgA and IgM responses show extensive inter-individual variability. On average, the levels of antistaphylococcal IgA and IgM increase from birth until the age of 2 years, whereas the levels of antistaphylococcal (maternal) IgG decrease. Placentally transferred maternal IgG does not protect against nasal colonization, whereas CHIPS, Efb, IsdA and IsdH are expressed *in vivo*, and therefore seem to play a role in nasal colonization of young children.

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Transparency Declaration

The Generation R Study is conducted by the Erasmus MC, Rotterdam in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, the Rotterdam Homecare Foundation, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. This work was supported by grants from the Erasmus MC, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (Zon Mw). Additionally, an unrestricted grant from Europe Container Terminals (ECT) Rotterdam funded this project. All authors declare that they have no conflicts of interest.

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