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Salivary antibody response to streptococci in preterm and fullterm children: A prospective study

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ABSTRACT

Objectives: Secretory immunoglobulins present in mucosa surfaces represent the first line of defense of the adaptive immune system against infectious challenges. Preterm (PT) neonates' humoral immunity is diminished compared to full-term (FT) newborns. The identification of important antigens (Ags) of virulence of oral species may help in the investigation of the mechanisms of antigenic stimulation and the development of the mucosal immune response. In the present study, we measured saliva levels of immunoglobulins A (IgA) and M (IgM) and characterized the specificity of IgA against Ags of several streptococcal species found early in life.

Methods: This was a prospective observational study. Salivary IgA (sIgA) antibody responses to bacterial species that are prototypes of pioneer (*Streptococcus mitis*, *S. sanguinis*, *S. gordonii*) and pathogenic (*Streptococcus mutans*) microorganisms of the oral cavity were studied in FT and PT children in two visits: at birth (T0) and at 3 months of age (T3). Salivas from 123 infants (72 FT and 51 PT) were collected during the first 10 h after birth (T0) and again at 3 months of age (T3). Salivary levels of IgA and IgM antibodies were analysed by enzyme-linked immunosorbent assay (ELISA). A subgroup of 26 FT and 24 PT children were compared with respect to patterns of antibody specificities against different streptococci Ags using Western blot assays.

Results: No significant differences ($P > 0.05$) in salivary levels of IgA and IgM between FT and PT babies were found at birth. At T3, mean sIgA values were similar between groups and sIgM levels were significantly higher in PT than FT ($P < 0.05$). Western blot assays identified positive IgA response to streptococci in the majority of children, especially in the FT group. There were some differences between groups in relation to the frequency of children with positive response to Ags and intensity of IgA response. In general, oral streptococci Ags were more frequently detected and bands were more intense in FT than in PT, especially in T3. Prospective analysis of patterns of sIgA against Ags of different streptococcal species revealed an increase in complexity of the sIgA antibody response from the first day of birth (T0) to T3 in PT and FT.

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Conclusion: The patterns of sIgA response to streptococci Ags appear to be influenced by the gestational age, which might reflect the level of immunological maturity of the mucosal immune system.

1. Introduction

The mucosal immune system represents the first line of defense of the adaptive immune response against infectious challenges. Antibacterial activity in secretions is mediated in part by secretory immunoglobulin A (SIgA) present in saliva and other body fluids.[1] The total levels of SIgA in saliva have been considered as an indicator of development of the mucosal immune system in children.[2,3] Transient reduction in levels of IgA detected in saliva was associated with increased susceptibility to infections of the gastrointestinal tract,[2,3] reinforcing the importance of the maturation mucosal immune susceptibility to infections.

There is some controversy about the ontogeny of the mucosal immune system, especially in the first months of life. Newborns have a higher incidence of colonization by several microorganisms when compared to adults or older children due to immaturity of the immune system.[4]

The oral cavity is the main entrance of microorganisms. *Streptococcus salivarius* (SSA) and *S. mitis* (SMI) represent the majority of bacteria that initially colonize the oral cavity.[5] Some of the pioneer species, such as SMI, *S. gordonii* (SGO), and SSA,[6,7] are associated with other diseases such as bacterial endocarditis. After the tooth eruption, new species are found in the mouth, such as *S. mutans* (SM).[8,9] SM can also be detected in children before tooth eruption.[10–12] SM is considered to be pathogenic for dental caries because of its ability to adhere and accumulate in the dental biofilm in the presence of sucrose and to produce and tolerate high concentrations of acids, which promote tooth demineralization.

The identification of important antigens (Ags) of virulence of oral species may help in the investigation of the mechanisms of antigenic stimulation on the mucosal immune response. The 153-kDa Ag of SGO was described as a glucosyltransferase (GTF) that may serve as a mechanism for colonization of the endocardium in infective endocarditis by mediating bacterial adhesion to human endothelium.[13] Studies in animal models showed that GTF production determined SGO ability to persist in the tooth surface biofilm.[14] Vacca-Smith et al.[15] purified a glucosyltransferase (GtfSs) from SSA with molecular weight of 170 kDa. IgA antibody levels to glucosyltransferase from SSA were detected in salivas of some children with 3–5 months of age.[16] Poulsen et al.[17] sequenced an IgA1 protease of SMI with 200 kDa. The 202-kDa Ag of SMI found in the present study also was detected in saliva samples of 6-month-old children.[18,19] Three main cell-associated Ags of SM have been shown to be involved in the capacity of these microorganisms to adhere and accumulate in the dental biofilm. These Ags include an adhesin (AgI/II) of 185 kDa, a GTF of 160 kDa, which synthesize glucan from sucrose, and a glucan-binding protein B (GbpB) with 56 kDa.[20] A previous study with Brazilian children of 5–11 months of age showed a high complexity of the salivary IgA response against Ags of SM,[18,21] indicating that the mucosal immune system is designed to respond to SM Ag challenge during the early stages of colonization. In addition, we have recently observed differences in neonatal IgA saliva levels and specificity to SM and SMI when full-term (FT) and preterm (PT) babies were compared.[22]

So, there is little information about the immune response during the early stages of bacterial challenge. It is possible that some factors can affect the initial response of pioneer species commensal of the oral cavity and may influence the patterns of immune response and the susceptibility to be colonized by other species, such as SM, later. In the present study, we analysed saliva levels of IgA and IgM and specificity of IgA against Ags of several streptococcal species at birth and after 3 months of age in FT and PT children.

2. Materials and methods

2.1. Study design

This is a prospective observational study performed at the *Hospital das Clinicas* of the School of Medicine of Ribeirão Preto, University of São Paulo, between October 2007 and May 2009. Fifty-one healthy PT newborns (<37 weeks of gestation) and 72 healthy FT newborns were included for the first evaluation of the study. Babies with congenital malformations, perinatal hypoxia, intracranial haemorrhage, with length or weight incompatible with gestational ages, and antibiotic therapy were excluded.

The prospective analysis of mucosal immune maturation was performed in 50 (26 FT and 24 PT) with similar levels of IgA, from 123 children, beginning at until 10 h of life (baseline, time zero (T0)) and 3 months thereafter (T3). The gestational age was calculated based on the first trimester ultrasound or through the newborn somatic evaluation using the Capurro et al.[23] method. The study was approved by the Ethics Committee of the Clinical of Hospital of the School of Medicine of Ribeirao Preto, University of São Paulo (Process number 2963/2007) and informed consent was obtained from the subjects' parents.

Patient demographic characteristics are listed in Table 1. Information on maternal and gestational background was obtained by interviewing the mother.

2.2. Collection of saliva

Samples of whole non-stimulated saliva were collected using sterile polypropylene transfer pipettes. Collections were performed in all children at approximately 4–10 h after birth