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Original article

Development and maturation of norovirus antibodies in childhood

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Abstract

The burden of norovirus (NoV) gastroenteritis is substantial in young children. Maternal antibodies are thought to protect a child from NoV infection in early infancy but subsequent development of NoV-specific protective immunity in children is still largely unexplored.

We have determined NoV-specific antibody seroconversion to GII.4 virus-like particles as an indicator of NoV infection in two children prospectively followed from birth to eight years of age. Blocking activity and affinity maturation of maternal and serum IgG antibodies were evaluated.

Our results show that multiple infections occur in children up to eight years of age. The titer, blocking activity and avidity of maternal antibodies determined susceptibility of an infant to NoV infection. NoV GII.4-specific antibodies with high blocking potential and avidity were developed at two to three years of age and were retained throughout the follow-up. Subsequent NoV infections may have contributed to the duration of protective NoV-specific immune responses that lasted for several years.

This study adds to current understanding of the duration of passive protection by maternal antibodies and the duration and quality of acquired immunity following primary and subsequent NoV infections in infants and young children, who are the main target group for NoV vaccine development.

Keywords: Norovirus; Infant; Children; Immunity; Infections; Serology

1. Introduction

Infants and children under five years of age are most susceptible to norovirus (NoV) acute gastroenteritis (AGE) [1–3], and are therefore one of the potential target groups for NoV vaccination. Young children have higher and longer viral shedding compared to adults [4] and may be considered as a pool for transmission of NoV to other vulnerable populations, e.g. senior citizens. NoV infects all age groups and in adults the NoV seropositivity is near 100% as a result of lifetime NoV exposure history [5].

Most NoVs infecting humans belong to genogroups GI and GII, which are genetically so distant that no intergenogroup cross-protective immune responses are generated [6]. However, after natural infection [7–11] and also vaccination [12] variable degree of intragenogroup cross-reactivity has been observed. NoV GII.4 genotype is responsible for

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approximately 55–80% of all NoV infections worldwide [13–15]. New epidemic antigenically distinct GII.4 variants emerge every two to three years [16–18] but considerable antibody cross-reactivity between GII.4 variants has been observed even in young children [19].

Correlates of protective immunity to NoV are not well established. A common finding has been that protection is not long lasting [5]. Early homologous challenge studies by Parrino et al. [20] and Johnson et al. [21] have shown that duration of protective immunity varies from two months to two years. There are a limited number of studies on NoV-specific immune responses in children. In contrast to those of adults, IgG responses to NoV during the first year of life are relatively weak and short-lived and the antibodies are of a low avidity [22,23]. Saito et al. [24] found that most infections in less than six-month-old infants are asymptomatic, probably due to maternally acquired antibodies and, possibly, breast-feeding. Altogether, the build-up of protective immunity after primary and secondary NoV infections is not well characterized.

NoV virus-like particles (VLPs) are commonly used for studying immune responses against NoV. The expression of the NoV capsid VP1 protein results in formation of VLPs that are morphologically and antigenically similar to native virions [25], thereby being also promising vaccine candidates [26,27]. Blocking assay has been used as a surrogate measure for NoV neutralization, determining the ability of serum antibodies to block binding of NoV VLP to its putative histo-blood group antigen (HBGA) receptors/attachment factors [12,28,29]. We have previously used NoV GII.4 VLPs to determine NoV seroprevalence in Finnish children [10]. Also, more recently an endpoint titer >51,200 and/or 90% blocking titer (BT_{90}) >100 was suggested as an indicator of protection from NoV infection in these children [10]. The recent challenge studies in adults have further affirmed correlation between the blocking antibodies and protection [12,30]. Antibody avidity is low in primary and recent NoV infections and increases with time [23], but the relevance of the Ab avidity in NoV protective immunity is not currently known.

In this study we determined seroconversion to NoV GII.4 VLPs as an indicator of NoV infection in two children prospectively followed from birth up to the age of eight years and examined the duration, blocking ability, and avidity of the antibodies.

2. Materials and methods

2.1. Study samples

Two healthy children (Subject 1 and 2) taking part in the Type I Diabetes Prediction and Prevention (DIPP) study [31] were prospectively followed for NoV GII.4-specific antibodies from birth to eight years of age. The DIPP study protocol was approved by the ethics committee of the Pirkanmaa Hospital District (Permit number: 97193M) and a written informed consent was obtained from the parents. Health records were collected during the follow-up visits and symptoms related to acute gastroenteritis episodes were recorded. Subject 1 had been breastfed exclusively for first five months and partially until the age of 25 months. Breastfeeding of the Subject 2 was exclusive for first two months, partial for one month. All procedures performed were conducted according to the principles expressed in the Declaration of Helsinki. Cord blood samples were taken at birth in year 2000 and thereafter blood samples were taken in sodium citrate tubes at the age of 3, 6, 12, 17 and 24 months and thereafter once per year up to eight years of age. Plasma fraction was stored at −70 °C until analyzed. Non-diarrheal stool samples were collected monthly at the age of 3–9 months and every two months until the age of 1 year, 9 months. Viral RNA was extracted from the stool suspensions and stored at −70 °C until reverse transcription-polymerase chain reaction (RT-PCR) and open reading frame 1 polymerase (region A) sequencing were used for NoV genotyping according to previously described methods [32].

2.2. Norovirus VLP production

GII.4-1999 capsid VP1 sequence originated from a patient sample collected in 1999 (GenBank reference strain accession no. AF080551) [23,33]. NoV GII.4-1999 VLPs used as antigens in analytical methods were produced by a baculovirus expression system (Invitrogen) in *Spodoptera frugiperda* (Sf9) insect cell cultures. VLPs were purified twice with discontinuous sucrose gradient ultracentrifugation as previously described [33]. The total protein concentration was quantified with Pierce® BCA Protein Assay (Thermo Scientific, Rockford). Protein purity, integrity, and morphology were determined by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blotting and transmission electron microscopy (EM) as described earlier [26,33].

2.3. Serum IgG ELISA

NoV GII.4-1999-specific IgG antibody levels were analyzed by ELISA as earlier described [10]. Serum specimens were diluted two-fold starting at 1:100 and plated on GII.4-1999 VLP coated (0.5 μg/ml in phosphate-buffered saline, PBS) 96-well half-area microtiter plates (Corning Inc., Corning, NY) blocked with 5% skimmed milk in PBS. Serum dilutions were incubated on plates for 1 h at 37 °C. Bound GII.4-1999-specific antibodies were detected with goat anti-human IgG-HRP (Invitrogen, CA, USA) followed by o-phenylenediamine (OPD) substrate (Sigma–Aldrich, MO, USA) and H_2O_2. Optical density (OD) was measured at λ 490 nm using the Victor2 1420 Multilabel Counter (Wallac, Perkin Elmer) plate reader. Background signal from the blank wells (wells without serum) was subtracted from all of the OD readings on the plate. Each plate contained NoV negative and positive control serum sample as an assay control. The cut-off value was determined as the mean OD reading of the negative control serum wells at a dilution 1:200 + 3 × standard error