Maternal vaccination is currently considered a strategy against respiratory syncytial virus (RSV) infections. In RSV-infected infants, high mucosal IgG levels correlated better with reduced RSV load and lower mucosal CXCL10 levels than plasma IgG levels. For future vaccination strategies against RSV, more focus should be on the mucosal humoral immune response.

The first description of respiratory syncytial virus (RSV) was published 60 years ago (1, 2). Despite the long awareness of the virus and the morbidity it causes, vaccines are still unavailable. This may partly be due to the results of one of the first vaccination trials in the 1960s, which had a devastating effect on RSV vaccine development. However, there are more hurdles to be encountered in RSV vaccine development. An important difficulty is the fact that the largest target group consists of very young infants (<6 months of age), who may respond inadequately to vaccination. Also, RSV is very efficient in evading the immune response, as is shown by the occurrence of reinfections throughout life, and last, there is no animal model that is fully permissive to human RSV infection.

Most vaccines aim to induce pathogen-specific IgGs. Palivizumab, a passively administered neutralizing monoclonal antibody, is able to protect infants from severe RSV disease (3–5). This shows that antibodies are able to prevent severe RSV infections and that induction of neutralizing antibodies by vaccination could potentially work. A vaccination route that is often considered for protection against RSV infection and which resembles passive immunization is maternal vaccination. High levels of maternally derived RSV-specific antibody, measured in the sera of infants, protect against RSV infection during the first months of life (6–8). Maternal vaccination aims to enhance the maternally derived IgG antibody levels in the infant.

However, it is unknown if plasma IgGs also reach the mucosal locations, if plasma and mucosal IgG levels are correlated with each other, and if plasma and mucosal IgGs are equally protective. To address these issues, we studied maternally derived preexisting RSV-specific plasma and mucosal antibody titers and their correlation with RSV load and RSV-associated inflammation, i.e., CXCL10, in a clinical pediatric cohort.

A total of 23 hospitalized children less than 3 months of age with laboratory-confirmed RSV infections were prospectively included during two consecutive winter seasons (November to April in 2010 to 2011 and 2011 to 2012). Patients with congenital heart or lung disease, immunodeficiency, or glucocorticoid use were excluded. Written informed consent was obtained from all parents of patients. The study was approved by the Central Committee on Research involving Human Subjects of the Radboud university medical center (Radboudumc). Demographics and clinical parameters were collected from questionnaires and medical records. Within 24 h after admission, a blood sample and a nasopharyngeal aspirate (NPA) sample were collected as previously described (9). The mean age of the patients was 53 days, the average gestational age was 38 weeks, and 48% of the patients were male (Table 1). Regarding their disease status, the mean duration of hospitalization was almost 10 days and the average RSV load gave a threshold cycle (Ct) value of 25. The young age of the infants enhanced the chance that this was their primary RSV infection; therefore, only maternal antibodies were studied. Moreover, it is known from the literature that infants do not mount significant neutralizing antibody responses before the age of 4 months (10).

Maternally derived RSV-specific IgGs were measured in both plasma and nasal aspirate samples from patients by enzyme-linked immunosorbent assay (ELISA). Whole RSV-A2 (4 × 10^7 fluorescent isothiocyanate [FITC]-detected infectious particles/ml) (diluted 1:200 in phosphate-buffered saline [PBS]; Lonza) was used to coat 96-well plates (Nunc Maxisorp). RSV-A2 was cultured and quantified as described previously (11). Plates were incubated for 5 h at 4°C, washed (PBS–0.05% Tween 20), and blocked for at least 2 h with 100 μl PBS–1% bovine serum albumin (BSA) (Sigma-Aldrich). A standard for IgG determination was prepared using two healthy volunteers. Samples were diluted 1:10 two times, as technical replicates, and incubated for 2 h at room temperature. After washing, alkaline phosphatase (AP)-conjugated antibody against human IgG (Southern Biotech) (diluted 1:800 in PBS–0.05% Tween 20 or PBS–0.05% Tween 20) was added and incubated for 2 h at room temperature. After washing, substrate solution (p-nitrophenyl phosphate) was added for 30 min at room temperature. The color reaction was stopped by the addition of 0.1 M NaOH. The plates were read at 405 nm. The absorbance at 405 nm was converted to RSV load using a standard curve established from serial dilutions of the virus.

Table 1: Patient characteristics

| Characteristic of RSV-infected patients (n = 23) | Value |
|-----------------------------------------------|-------|
| Median days of age (IQR)*                     | 53 (31–70) |
| No. (% ) of males                             | 11 (48) |
| Median gestational wks of age (IQR)           | 38.4 (38.3–38.5) |
| Median RSV load (Ct value) (IQR)              | 24.8 (24.6–25.0) |
| Median days of hospitalization (IQR)          | 9.6 (9.4–9.7) |

* IQR, interquartile range.
1:10,000 in 1% BSA) was added and incubated for 2 h at room temperature. After washing, substrate buffer (10 mM diethanolamine–0.5 mM MgCl₂) was added. Absorbance was measured at 450 nm and 690 nm after 30 min and 60 min. Background binding was subtracted from each sample measurement, and results were calculated using arbitrary units (AU). In our pediatric cohort, no correlation was found between the levels of mucosal and plasma IgGs (Fig. 1A), suggesting that, in addition to passive transudation, other mechanisms are at play during infection. It has been shown that, due to an infection, IgG antibodies can be actively secreted to the lumen (12).

For viral diagnostics, samples were analyzed by multiplex PCR,
quantifying 15 different viral pathogens, as previously described (13). In contrast to plasma IgG levels (Fig. 1B), mucosal IgG levels were correlated with the RSV load; higher mucosal IgG levels resulted in a lower RSV load (Fig. 1B). This shows that mucosal IgG levels are a better correlate than plasma IgG levels for viral load.

High CXCL10 plasma levels are indicative of RSV-associated inflammation (14). Therefore, we tested whether mucosal and plasma CXCL10 levels correlated with RSV load. The concentration of CXCL10 was determined using a cytometric bead array (CBA), according to the manufacturer’s protocol (BD Biosciences). Briefly, CXCL10 levels in individual plasma samples (50 μl) were analyzed, in duplicate, using the CBA kit and an LSR II flow cytometer. We found that a higher viral load resulted in higher mucosal CXCL10 levels but not in higher plasma CXCL10 levels (Fig. 1C). Therefore, mucosal CXCL10 is a better correlate for viral load than plasma CXCL10.

Finally, mucosal CXCL10 levels were correlated with plasma and mucosal IgG levels. We found a significant correlation showing that higher mucosal IgG levels resulted in lower mucosal CXCL10 levels (Fig. 1D). No correlation was found between mucosal CXCL10 and plasma IgG levels. This suggests that mucosal antibodies also reduce RSV-associated inflammation. As a control for confounders, none of the measured parameters were correlated with the age or gender of the infants (data not shown).

IgA is the predominant immunoglobulin present in the mucosa; therefore, not many studies have focused on the presence and function of IgG at this location. However, as maternal vaccination aims to enhance mainly the IgG levels of the infant, it is of importance to study whether maternally derived IgGs are present on the nasopharyngeal mucosa of the infant and, if so, whether that presence correlates with viral load and the immune response. Our data suggest that high levels of IgG on the nasal mucosa are able to protect against RSV infections. Although plasma IgG levels are often used as a readout for vaccine development, it should be taken into account for future vaccine development that mucosal IgG levels are potentially of greater importance than plasma IgG levels. These results suggest that mucosal (intranasal) vaccination which aims to evoke a strong mucosal immune response (15) may be a more effective vaccination strategy. For future studies, a group with very mild RSV infection should be included to determine what level of mucosal IgGs may protect against severe infection. Moreover, correlating maternal plasma IgG levels with mucosal IgG levels of the infant would give insight into the potential of maternal vaccination. Also, more knowledge has to be generated as to how IgG molecules are transported to the nasopharynx and whether this can be enhanced. This could lead to novel immunization strategies to improve mucosal protection by maintaining long-lasting higher IgG levels in the nasopharynx.

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