Vaccine 33 (2015) 1797–1801

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Quantifying maternally derived respiratory syncytial virus specific neutralising antibodies in a birth cohort from coastal Kenya

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ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form 2 January 2015
Accepted 16 February 2015
Available online 26 February 2015

Keywords:
Vaccine design
Respiratory syncytial virus
Maternal antibody
Birth cohort
Neutralisation
Rate of decay

ABSTRACT

Background: Severe respiratory syncytial virus (RSV) disease occurs predominantly in children under 6 months of age. There is no licensed RSV vaccine. Protection of young infants could be achieved by a maternal vaccine to boost titres of passively transferred protective antibodies. Data on the level and kinetics of functional RSV-specific antibody at birth and over the early infant period would inform vaccine product design.

Methods: From a birth cohort study (2002–2007) in Kilifi, Kenya, 100 participants were randomly selected for whom cord blood and 2 subsequent 3-monthly blood samples within the first year of life, were available. RSV antibodies against the A2 strain of RSV were assayed and recorded as the logarithm (base 2) plaque reduction neutralisation test (PRNT) titre. Analysis by linear regression accounted for within-person clustering.

Results: The geometric mean neutralisation antibody titre was 10.6 (SD: 1.13) at birth with a log-linear decay over the first 6 months of life. The estimated rate of decay was −0.58 (SD: 0.20) $\log_2$PRNT titre per month and a half-life of 36 days. There was no significant interaction between cord titre and rate of decay with age. Mean cord titres rose and fell in a pattern temporally tracking community virus transmission.

Conclusions: In this study population, RSV neutralising antibody titres decay approximately two-fold every one month. The rate of decay varies widely by individual but is not related to titre at birth. RSV specific cord titres vary seasonally, presumably due to maternal boosting.

1. Background

Severe RSV disease occurs primarily in infancy and predominantly among children 1–5 months of age [1]. Globally, RSV disease may be responsible for nearly 200,000 deaths per year in young children and 99% of these deaths occur in low income countries [2]. An annual rate of hospitalisation with RSV associated pneumonia of 1–2 per 100 for children in the first year of life has been reported in a rural community in Coastal Kenya [1,3].

Prevention of RSV disease is primarily targeted towards young infants [4], for which two vaccine strategies are appropriate: first, to vaccinate infants at the earliest age when able to develop a protective immune response with minimal reactogenicity, and second, to boost the level of RSV-specific antibodies in pregnant women before delivery to extend the duration of protective antibodies in early infancy [5–7].

There is evidence which supports the idea that maternal specific RSV antibodies provide protection from RSV disease [7,8]. Glezen et al. demonstrated that infants born with higher levels of antibody develop infection at a later age, and infants infected in the presence of moderate levels of serum antibody have milder illnesses than infants infected with lower or undetectable levels of antibody [7]. A case-control study in rural Mozambique showed that high levels of antibodies of maternal origin were associated with protection against RSV disease [5]. A randomised double blind placebo controlled trial among premature infants and infants with bronchopulmonary dysplasia showed that monthly prophylaxis with

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http://dx.doi.org/10.1016/j.vaccine.2015.02.039

Palivizumab (a humanised monoclonal IgG antibody) was associated with a 55% reduction in hospitalisation as a result of RSV [9]. In addition, there are data that suggest a defined level of neutralising antibody which protects against RSV disease [10].

The success of a maternal RSV vaccination strategy will be governed by the degree to which a vaccine will boost levels of protective antibodies transferred by the mother to the infant and by the rate at which those antibodies decay. In this study we provide baseline data necessary to evaluate vaccine potential. Specifically, in a birth cohort of infants from Kilifi in Kenya, we estimate the mean of and variation in RSV neutralising (i.e. functional) antibody in newborn cord blood, and its rate of decay in relation to starting titre.

2. Methods

2.1. Study site and population

This study was conducted at the Kenya Medical Research Institute (KEMRI)—Wellcome Trust Collaborative Research Programme, in Kilifi, coastal Kenya [11]. Between 1999 and 2007, a birth cohort study [12,13] was conducted to investigate susceptibility to invasive pneumococcal disease among children aged 0 to 23 months. This study was a natural history study, with no intervention, in which cord and 3 monthly blood samples were collected for each participant.

The current study used archived serum samples of children who were participants of the Kilifi Birth Cohort (KBC) study and who were residents of the Kilifi Health and Demographic Surveillance System (KHDSS). Characteristics of this study population have been described before [11,12,14].

Approximately 6000 KBC study participants were recruited in the maternity ward and at the maternal and child health clinic at Kilifi District Hospital (KDH). A random sample of 100 participants from the KBC study, recruited from the KDH maternity ward, were selected from the study database regardless of RSV disease. Each selected participant had at least 3 blood samples (specifically, a cord blood and two follow up samples) each separated by approximately 3 months.

The temporal pattern of cases of RSV associated severe pneumonia was derived from continuous surveillance of children under 5 years of age admitted to Kilifi District Hospital, Kilifi, which serves the KHDSS population [1,15]. An RSV epidemic was empirically defined to begin when at least 10% of tested samples were RSV positive or with the observation of at least 2 cases of RSV infection per week in each of 2 consecutive weeks and were defined to continue as long as these conditions were satisfied, with the requirement that an epidemic must last for ≥4 weeks as previously defined [1]. These data were used to compare with the temporal pattern of cord titres.

The Kenya National Ethical Review Committee approved this study.

2.2. Laboratory procedures

Archived serum samples had been stored at −80 °C. The titre of RSV neutralising antibodies was determined by a plaque reduction neutralisation test (PRNT) as described previously [16]. The method incorporated a step in which serum samples were incubated at 56 °C in a water bath for 30 min to inactivate complement cascade proteins. The dilution of a test serum sample required to induce 50% neutralisation of a known titration of RSV A2 virus was determined using the Spearman Karber method [16].

2.3. Statistical analyses

Data analysis was conducted using STATA version 11.2 (College Station, Texas). The laboratory data were merged with archived data from the KBC and KHDSS databases for analysis. Sample PRNT titres were logarithmically transformed (base 2) for all statistical analyses. The titre of cord, first and second samples for an individual were defined as TC, T1 and T2, respectively. To offset bias on the rate of decay arising from RSV infection, we applied the following criteria. For individuals with T1 ≥ TC, all results for that individual were excluded, and for individuals with T2 ≥ T1 the result for sample T2 was excluded. In addition, all samples collected at ages ≥7 m after birth were excluded due to there being few in number and of wide age range (7–11 m) and a high likelihood that the measured antibody would be active rather than passive. The rate of decay of RSV specific $\log_2$PRNT titres from birth to <7 months of life was determined by simple linear regression, accounting for clustering of titres for samples from the same individual using the procedure for Huber–White sandwich estimator. Possible interaction between cord antibody titre and rate of decay of maternal antibodies was tested using the model with individuals categorised by quartile of titre at birth. Age dependence in the rate of decay of antibody titres was evaluated by comparing model fits using linear and quadratic terms for age. The Wald test was used to evaluate the significance of removal of variables in nested models. To evaluate the effect of infection on the antibody levels, the rate of decay of those participants with at least 2 samples collected within an RSV epidemic was estimated and compared to that of participants with at least 2 samples collected outside an RSV epidemic. A two sample *t*-test was used to compare the levels of neutralising antibodies and the mean of the rates of decay between samples collected within and outside an RSV epidemic and between binary covariates for birthweight (low birth weight <2.5 kg) measured using a weighing scale at birth and gestation period (premature < 37 weeks) based on date of last menstruation or by clinical evaluation.

3. Results

A total of 300 samples from 100 cohort participants were selected. For 8 individuals, the second follow-up sample (T2) was not available, therefore, 292 samples were screened for RSV specific neutralising antibodies. Applying the exclusion criteria as described in the Methods, 18 additional samples were removed (9 samples with T2 ≥ T1 and 9 samples ≥7 months), leaving 274 samples from 100 participants (76 with 3 samples, 22 with 2 samples and 2 with 1 sample) for analysis.

The frequency distribution of log-transformed PRNT titres for cord blood samples was approximately normal (Fig. 1) with mean concentration 10.6 (95% Confidence Interval (CI) 10.3–10.7, variance 1.28) and median of 10.6 (Interquartile Range (IQR) 9.95–11.4, 10th percentile 8.87 $\log_2$PRNT). The mean birth weight in kilograms of the participants in this study was 2.89 kg (SD: 0.49) and 19% were born with low birthweight <2.5 kg (mean: 2.16; SD: 0.21). Only 63 participants had data on gestational period. The mean gestation period in weeks was 38.6 (SD: 3.16). Of the 63 individuals with gestational data, 15(24%) were born prematurely i.e. <37weeks (mean 34.4; SD: 2.48). There was a significant difference in the cord blood neutralising antibody concentrations among the low birth weight, 9.9 $\log_2$PRNT compared to the normal weight 10.7$\log_2$PRNT ($P = 0.02$, $t = -2.37$) and between premature, 9.8 $\log_2$PRNT and not premature, 10.9 $\log_2$PRNT ($P = 0.002$, $t = -3.18$).

The distribution of log-transformed PRNT titres by age is shown in Fig. 2, with the best fit linear regression model (age range 0–<7 m) giving an average reduction per month in $\log_2$PRNTof −0.58 (95%CI: −0.65 and −0.51), and a predicted mean titre at birth of 10.5 (95%CI