Short communication

The association between age and the development of respiratory syncytial virus neutralising antibody responses following natural infection in infants

Charles J. Sande [a],[*],[1], Patricia A. Cane [b], D.J. Nokes [a],[c]

[a] KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya
[b] Public Health England, London, United Kingdom
[c] School of Life Sciences and WIDER, University of Warwick, Coventry, United Kingdom

ARTICLE INFO

ABSTRACT

To determine the age at which infants mount significant neutralising antibody responses to both natural RSV infection and live vaccines that mimic natural infection, RSV-specific neutralising antibodies in the acute and convalescent phase sera of infants with RSV infection were assayed. Age-specific incidence estimates for hospitalisation with severe RSV disease were determined and compared to age-specific neutralising antibody response patterns. Disease incidence peaked at between 2 and 3.9 months of life. Following natural infection, relative to the mean acute phase antibody titre, the mean convalescent titre was lower in the 0–1.9 month age class, no different in the 2–3.9 month age class and greater in all age classes greater than 4 months. These data suggest effective vaccination with live vaccines that mimic natural infection may not be achieved before the age of 4 months. Maternal vaccination may be an alternative to direct infant vaccination in order to protect very young babies.

1. Introduction

RSV is an important cause of acute lower respiratory infection in infants and elderly adults [1]. Recent estimates have shown the considerable global burden of RSV-associated disease [2] and have highlighted the need for the development of effective vaccines for use in vulnerable populations. Severe RSV infection in infants can result in the development of potentially life-threatening severe pneumonia [3] and is increasingly being recognised as predisposing to severe pneumonia in the short term [4] and as a risk factor for the development of wheeze and asthma in later life [5]. Previous studies have provided evidence that immunity from lower respiratory tract disease correlates with levels of neutralising antibodies [6,7], an assertion that is supported by the clinical experience with the prophylactic neutralising monoclonal antibody, Palivizumab, which has been associated with up to a 78% reduced risk of hospitalisation among premature infants [8].

The key target group for vaccination against RSV is infants under the age of 6 months in whom the risk of severe disease is greatest. The prospect of active immunisation of this population is hindered by safety concerns related to the administration of non-replicating vaccines which are associated with potentiation of disease upon re-exposure in both infants [9] and animals [10]. In contrast, replicating vaccines such as live-attenuated vaccines have been shown in several clinical trials to have a relatively good safety profile [11,12] and are thought to be the safest alternative for providing direct protection for infants. RSV vaccine development faces the additional challenge of vaccinating infants at an age that is associated with both a high prevalence of maternally derived antibodies as well as relative immunological immaturity. The association between age and the neutralising response to natural RSV infection in infants is therefore an important consideration in the development of live-attenuated vaccines, whose antigenic profile is thought to closely mirror that of wild type virus and which might therefore be expected to induce responses that broadly resemble natural infection responses. This study investigated the development of neutralising antibody responses generated upon natural infection in early infancy. The implications of the results on infant vaccination strategy are discussed.

* Corresponding author at: Kenya Medical Research Institute (KEMRI)/Wellcome Trust Research, Centre for Geographic Medicine Research (Coast), P.O. Box 230, 80108 Kilifi, Kenya. Tel.: +254 41 7522535/7522063/7525043; fax: +254 42 7522390.
E-mail addresses: csande@kemri-wellcome.org,
CSande@kilifi.kemri-wellcome.org (C.J. Sande).
[1] Present address: Oxford Vaccine Group, University of Oxford, Old Road, Headington, Oxford OX3 7LE, United Kingdom. Tel.: +44 01865 857420.
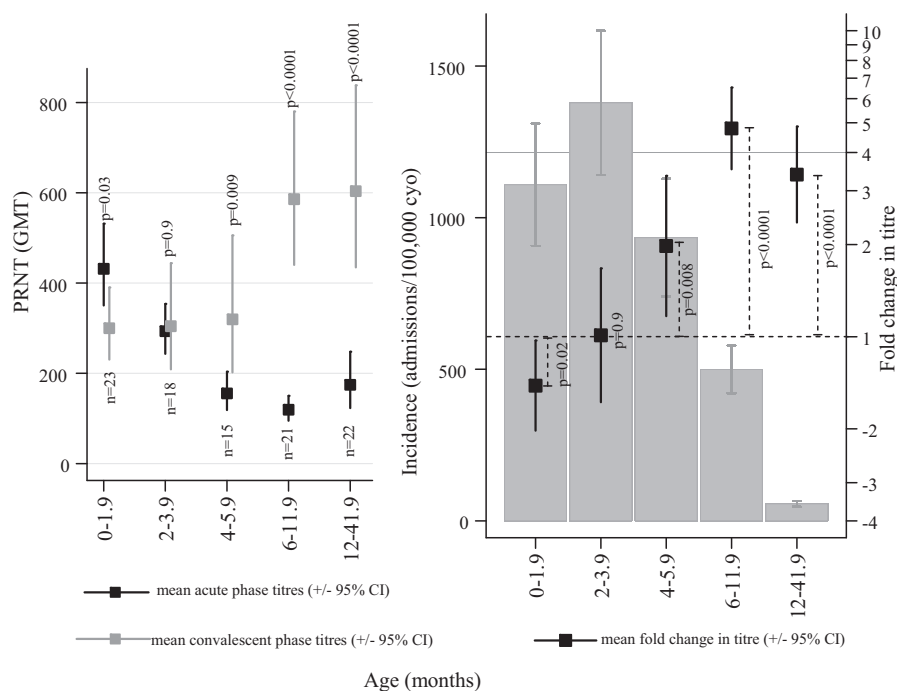
Fig. 1. (a) The relationship between acute and convalescent phase titres with age. Mean acute phase titres are depicted by the black filled boxes (with corresponding 95% confidence intervals) while mean convalescent phase titres are shown in grey filled boxes (with 95% confidence intervals). The *p* values above each mean acute/convalescent pair indicate whether the mean differences in titre are statistically significant. The values below the acute/convalescent pair are the number of individuals in each age class whose acute/convalescent responses were analysed. (b) Comparison between age-specific incidence estimates of admission with severe disease and the ability to generate a neutralising antibody response following natural infection. The incidence of admission with RSV associated pneumonia within the Kilifi Health and Demographic Surveillance System (with corresponding 95% confidence intervals) is shown in the grey bars while the mean fold change in neutralising antibody titre from the acute to convalescent phases of infection are depicted using the black filled boxes (with corresponding 95% confidence intervals). The horizontal dashed line indicates a 1-fold change in titre; above which a rise in titre is observed and below which a decline in titre is observed. The solid horizontal line indicates the 4-fold level that is used to define a seroconversion. The *p* values against the vertical dashed lines connecting the mean fold changes in titre to the horizontal dashed line indicating a 1-fold change in titre indicate whether the age-specific mean fold changes in titre are significantly different from 1. The association between age-specific incidence estimates of disease and fold changes in titre from the acute to convalescent phases of infection is not causal.

2. Materials and methods

The study was set in the Kilifi District Hospital (KDH) on the coast of Kenya [14]. Acute and convalescent phase sera, collected at admission and approximately 4 weeks after admission, respectively, were obtained from 99 patients aged 6 days to 41 months who were admitted to KDH with severe RSV infection. RSV diagnosis was done using an immunofluorescent antibody test on nasopharyngeal samples [13]. Neutralising antibodies to the A2 strain of RSV were measured by a previously described microplaque reduction neutralisation assay [15]. Written informed consent was sought from children's parents while ethical approval for the study was granted by the Kenya Medical Research Institute Ethical Review Committee.

Data were analysed using Stata (StataCorp, Texas). For the estimation of both disease incidence and antibody response, data were stratified in five age classes: 0–1.9, 2–3.9, 4–5.9, 6–11.9 and 12–41.9 months of age. Age-specific incidence estimates for admission with severe RSV pneumonia were calculated for the period January 1st 2002 to December 31st 2008, by dividing the number of pneumonia admissions resident in KHDSS with a laboratory diagnosis of RSV by the resident population size at the midpoint of the study period [13]. The difference between the mean acute and convalescent phase titres in different age classes was tested using a paired *t* test. To determine whether a significant response to infection was generated following infection, the magnitude of the neutralising antibody response – expressed as fold change in titre from the acute to convalescent phases of infection – was compared to a value of 1 using an unpaired *t* test.

3. Results

In the first year of life there was a progressive decline in the titre of acute phase neutralising antibodies, which coincided with an increase in convalescent titres over the same period (Fig. 1a). The incidence of severe RSV associated pneumonia during the study period rose sharply after birth; starting at 1108 admissions/100,000 child years of observation (cyo) at between 0 and 1.9 months of age (95% CI: 906–1310) and peaking at 1378 admission/100,000 cyo (95% CI: 1140–1616) at between 2 and 3.9 months of age. The incidence of severe RSV pneumonia thereafter declined to 934 admissions/100,000 cyo (95% CI: 740–1128) in the 4–5.9 month age class, and was lowest in the 6–11.9 and 12–41.9 month age classes at 499 admissions/100,000 cyo (95% CI: 420–578) and 56 admissions/100,000 cyo (95% CI: 46–65), respectively, as shown in Fig. 1b. In the first year of life the response to infection, measured as fold change in neutralising antibody titre from the acute to convalescent phases of infection, increased progressively with age. In the first 2 months of life (0–1.9 months), there was a significant decline in the neutralising response, *i.e.*, fold change less than unity ($p = 0.02$; Fig. 1), while no significant change in titre was observed at 2–3.9 months of age ($p = 0.1$). However, as shown in Fig. 1b, in all age classes of children older than 4 months of age, there was a significant rise in the titre of neutralising antibodies following natural infection.

The proportion of infants who had a detectable rise in titre from the acute to convalescent phases of infection (fold change in titre >1) increased with age as shown in Fig. 2. In the youngest age class (0–1.9 months old), only 26% of infants with a confirmed RSV
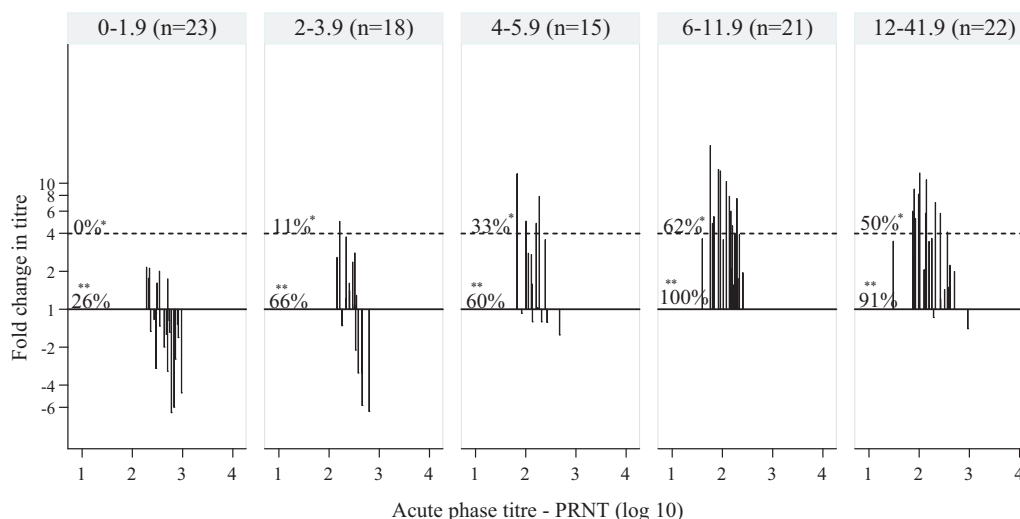
Fig. 2. The increase in the magnitude of the neutralising antibody response with age. The percentage of all infants within specific age classes with any rise in titre (**) and the proportion of infants who seroconverted (*) following natural infection is shown. The vertical lines indicate the fold change in titre from the acute to convalescent phases of infection. Each panel header indicates the age class being analysed as well as the number of individuals in that age class. Responses greater than unity indicate rises in titre following infection, while those below indicate decline in titre. Responses that are equivalent to or greater than a 4-fold rise in titre (dashed line) are considered to be seroconversions.

infection had a rise in titre following infection. In subsequent age classes, the proportion of infants with a detectable rise in the titre of neutralising antibodies following infection rose sharply with age, reaching 66% in the 2–3.9 month age class and 60% in the 4–5.9 month age class. The greatest response was observed in the 6–11.9 month age class where all infants had detectable rises in titre following infection. The same trend was observed when the data were analysed in terms of infants who generated an antibody response that reached or exceeded the 4-fold seroconversion threshold. No seroconversions were observed in the youngest age class (0–1.9 months old). However in subsequent age classes the rate of seroconversion steadily increased with age. Seroconversion rates in the 2–3.9, 4–5.9, 6–11.9 and 12–41.9 months of age were 11%, 33%, 62% and 50% respectively.

4. Discussion

In the current study, age-specific neutralising antibody response patterns to natural infection were determined among infants of different ages in order to provide an accurate estimate of the youngest age at which infants mount robust neutralising antibody responses. In contrast to the significant increases in the neutralising response observed among infants who were above 4 months of age, there was, a significant decline in the neutralising antibody response in the 0–2.9 month age class, while in the 2–3.9 month age class, where disease burden was greatest, there was no significant change in titre following infection. Previous work has suggested that infants under the age of 6 months, generally mount poor responses to infection [16], an effect that is not linked to age *per se*, but rather to the titre of pre-existing antibodies at the time of infection [17]. This poor responsiveness is postulated to be due to suppressive effects of maternally derived antibodies by mechanisms such as epitope masking and Fc receptor mediated phagocytosis of antibody–virus complexes [18]. The data presented here suggest that as a result of passive maternal antibody decline, these suppressive effects are sufficiently diminished by around 4 months of age, to allow for the detection of significant infant responses to infection. The responses presented in this paper are presumed to be representative of the general infant population who predominantly suffer mild disease. Similar studies in infants with mild disease should be the subject of future research in order to establish the validity of this extrapolation. The disease incidence estimates presented in Fig. 1b, suggest that in order to have the greatest impact on disease burden, infants should be vaccinated prior to the period of greatest risk of disease, at about 2 months of age. However the poor response to natural infection in infants under the age of 4 months suggests that such infants are unlikely to mount strong neutralising antibody responses to live vaccines. Nonetheless, the data presented suggest that vaccination of infants aged 4 months and above is likely to provide substantial benefit. To protect very early infants at the period of greatest risk, there is need to explore alternative strategies such as maternal vaccination. The boosting of the titre of trans-placentally transferred antibody will increase the duration of infant protection and delay the age of first infection, at which time infection is less likely to result in severe disease [19]. Recent studies [20,21] show that some vaccines that are designed for maternal vaccination are both protective in animals and have a good safety and immunogenicity profile in healthy adults, providing some basis to suggest that this might be a viable alternative to the direct vaccination of the young infant or suit a combined strategy of maternal vaccination followed by delayed later infant active immunisation.

Conflicts of interest

All authors declare that there is no conflict of interest.

Author contributions

CJS, PAC and DJN were involved in study design, statistical analyses, interpretation of the data and writing of the manuscript. CJS carried out the laboratory assays.

Approval

All authors have provided approval for publication of this article. This paper is published with the approval of the Director, KEMRI.

Funding

had no role in the design of the study, data collection, analysis and interpretation.

References

[1] Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005;352:1749–59.

[2] Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010;375:1545–55.

[3] Johnson JE, Gonzales RA, Olson SJ, Wright PF, Graham BS. The histopathology of fatal untreated human respiratory syncytial virus infection. Mod Pathol 2007;20:108–19.

[4] Munywoki PK, Ohuma EO, Ngama M, Bauni E, Scott JA, Nokes DJ. Severe lower respiratory tract infection in early infancy and pneumonia hospitalizations among children, Kenya. Emerg Infect Dis 2013;19:223–9.

[5] Blanken MO, Rovers MM, Molenaar JM, Winkler-Seinstra PL, Meijer A, Kimpen JL, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. N Engl J Med 2013;368:1791–9.

[6] Glezen W, Paredes A, Allison J, Taber L, Frank A. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. J Pediatr 1981;98:708–15.

[7] Piedra PA, Jewell AM, Cron SG, Atmar RL, Glezen WP. Correlates of immunity to respiratory syncytial virus (RSV) associated-hospitalization: establishment of minimum protective threshold levels of serum neutralizing antibodies. Vaccine 2003;21:3479–82.

[8] The IMpact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics 1998;102:531–7.

[9] Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am J Epidemiol 1969;89:422–34.

[10] Murphy BR, Sotnikov AV, Lawrence LA, Banks SM, Prince GA. Enhanced pulmonary histopathology is observed in cotton rats immunized with formalin-inactivated respiratory syncytial virus (RSV) or purified F glycoprotein and challenged with RSV 3–6 months after immunization. Vaccine 1990;8:497–502.

[11] Wright PF, Karron RA, Belshe RB, Thompson J, Crowe Jr JE, Boyce TG, et al. Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine candidate in infancy. J Infect Dis 2000;182:1331–42.

[12] Wright PF, Karron RA, Belshe RB, Shi JR, Randolph VB, Collins PL, et al. The absence of enhanced disease with wild type respiratory syncytial virus infection occurring after receipt of live, attenuated, respiratory syncytial virus vaccines. Vaccine 2007;25:7372–8.

[13] Nokes DJ, Ngama MJ, Bett A, Abwao J, Munywoki P, English M, et al. Incidence and severity of respiratory syncytial virus pneumonia in rural Kenyan children identified through hospital surveillance. Clin Infect Dis 2009;49:1341–9.

[14] Scott JA, Bauni E, Moisi JC, Ojal J, Gatakaa H, Nyundo C, et al. Profile: The Kilifi Health and Demographic Surveillance System (KHDSS). Int J Epidemiol 2012;41:650–7.

[15] Sande CJ, Mutunga MN, Medley GF, Cane PA, Nokes DJ. Group and genotype specific neutralising antibody responses against respiratory syncytial virus (RSV) in infants and young children with severe pneumonia. J Infect Dis 2012.

[16] Brandenburg A, Groen J, Steensel-Moll H, Claas E, Rothbarth P, Neijens H, et al. Repiratory syncytial virus specific serum antibodies in infants under six months of age: limited serological response upon infection. J Med Virol 1997;52:97–104.

[17] Shinoff JJ, O'Brien KL, Thumar B, Shaw JB, Reid R, Hua W, et al. Young infants can develop protective levels of neutralizing antibody after infection with respiratory syncytial virus. J Infect Dis 2008;198:1007–15.

[18] Crowe Jr JE. Influence of maternal antibodies on neonatal immunization against respiratory viruses. Clin Infect Dis 2001;33:1720–7.

[19] Ohuma EO, Okiro EA, Ochola R, Sande CJ, Cane PA, Medley GF, et al. The natural history of respiratory syncytial virus in a birth cohort: the influence of age and previous infection on reinfection and disease. Am J Epidemiol 2012;176:794–802.

[20] Smith G, Raghunandan R, Wu Y, Liu Y, Massare M, Nathan M, et al. Respiratory syncytial virus fusion glycoprotein expressed in insect cells form protein nanoparticles that induce protective immunity in cotton rats. PLoS ONE 2012;7:e50852.

[21] Glenn GM, Smith G, Fries L, Raghunandan R, Lu H, Zhou B, et al. Safety and immunogenicity of a Sf9 insect cell-derived respiratory syncytial virus fusion protein nanoparticle vaccine. Vaccine 2013;31:524–32.