LETTER TO THE EDITOR

Impact of CD14 promoter variants on measles vaccine responses and vaccine failure in children from Australia and Mozambique

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Conflict of interest

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Considering the high burden of measles disease, including the increase in measles incidence and outbreaks over the last few years (1), the global annual mortality rates of ~158,000 (1), and the measles vaccine failure rates of > 30% in underdeveloped countries (2), it is important to identify the factors that cause poor responses to measles vaccine. Host genetics are likely to play a crucial role (3), particularly variants that alter key innate immune response genes.

The immune development of infants may be affected by early life exposure to bacterial antigens, as Th1 maturation is enhanced by microbial stimulation (4). This priming of the immune system may be affected by the CD14 receptor, a critical component in the response to bacterial lipopolysaccharide (LPS), via the toll-like receptor-4 (TLR4) pathway (5). There are several common variations in the CD14 gene, and these CD14 variants can functionally affect soluble (s)CD14 levels, which in turn can alter the Th1/Th2 cytokine balance (6). Whether these CD14 variants impact early innate immune responses and the development of primary vaccine responses, and contribute to vaccine failure, is not yet known.

We investigated common CD14 single nucleotide polymorphisms (SNPs), their functional effects on sCD14 levels, and their associations with measles IgG responses and measles vaccine failure. We assessed these outcomes in two differing cohorts of measles-vaccinated children from Perth, Western Australia and Manhiça, Mozambique, Africa. To our knowledge, this is the first study to investigate CD14 variants with regards to measles vaccine responses. This study is also unique with regards to our resident African population, some of whom displayed true measles vaccine failure.

Healthy 12-14 month old Caucasian children were recruited from Perth, Western Australia (n=137), and each received a single dose of measles-mumps-rubella (MMR) vaccine (Priorix™; GlaxoSmithKline, Belgium) (7,8). Children aged 6 months-14 years were also recruited from Manhiça, Mozambique, Africa (9,10), some of whom presented with the clinical symptoms of measles despite vaccination (vaccine failure cases n=66), and were compared with age-matched controls (n=172). Children were designated as
measles vaccine failure cases if they had been vaccinated (recorded by vaccination card) and they presented with the clinical symptoms of measles (presence of fever, rash and one or more of the following signs: cough, coryza or conjunctivitis) (9,10).

DNA was extracted from blood using Qiagen QIAamp DNA Mini kits. Three polymorphisms in CD14 (-159C/T, -550G/A, -1619C/T) were genotyped using an iPLEX assay on a MALDI-TOF MassARRAY platform (Sequenom Inc., San Diego, CA, USA). Haplotypes were inferred using the Bayesian statistical based program PHASE (11). Soluble (s)CD14 levels were quantified in plasma in pre- and (6-8 weeks) post-vaccination samples of the Australian cohort using Quantikine Human sCD14 Immunoassay (R&D Systems). Post-vaccination measles-specific IgG and IgM titres were measured by Enzygnost Immunoassay kits (Dade Behring, Marburg, Germany) and in-house ELISA, respectively (7-10). All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Chi-squared tests were used to ensure that genotype frequencies did not deviate from the Hardy-Weinberg equilibrium, to compare allele frequencies between the two populations, and to determine the proportion of genotypes in the Mozambique vaccine failure cases compared with controls. Differences between antibody titres with genotype were analysed by one-way ANOVA or nonparametric Kruskal-Wallis tests, with Pearson’s correlations and linear regression used when correcting for confounders (age, gender, time since vaccine, age when vaccinated).

All CD14 polymorphisms had significantly different allele frequencies between the two populations (P<0.0001; Table 1). Post-vaccination sCD14 levels were significantly lower (mean 1.63µg/ml, 95% CI: 1.54 – 1.73) compared with pre-vaccination sCD14 levels (2.12µg/ml, 2.02 – 2.22; P<0.001) (Figure 1A). CD14 -159C/T and -550G/A were not associated with either pre- or post-vaccination sCD14 (Figure 1B). However, CD14 -1619TT had significantly lower post-vaccination sCD14 (1.98µg/ml, 1.82 – 2.13) compared with CC (2.39µg/ml, 2.08 – 2.69) and CT (2.18µg/ml, 2.02 – 2.33; P=0.029) (Figure 1B). CD14
polymorphisms were not significantly associated with measles-specific IgG production in either cohort (Table 1). Haplotype analysis also showed no significant associations (data not shown).

A subset of Mozambique children were designated as “clinically-defined” measles vaccine failure cases (n=66 total, n=18 with samples) as they presented with measles symptoms and had documented vaccination history. Measles IgG was not different between these cases compared with age-matched controls (n=71) (P=0.111). When taking into account laboratory measles confirmation via IgM, subjects were also grouped into “laboratory-defined” cases (n=9) and controls (n=165) and measles IgG was significantly lower in these cases compared with controls (P=0.004) (10). CD14 polymorphisms did not show different distributions between clinically- or laboratory-defined vaccine failure cases and controls (Table 1).

In this study, we report that although CD14 polymorphisms can functionally affect sCD14 levels and therefore can presumably influence early life immune responses and immune maturation, there was no evidence to suggest that these variants have a significant impact on measles vaccine responses or vaccine failure in children from Australia or Mozambique.

CD14 has previously been shown to be involved in the antiviral response to respiratory syncytial virus, a single stranded RNA paramyxovirus closely related to measles (12), and CD14 polymorphisms have also previously been linked with pneumococcal vaccine responses (13), however this is the first time this gene has been investigated with regards to measles vaccine. Our results with sCD14 levels both contrast and agree with previous studies. CD14 -159C/T has been linked with levels of sCD14, and sCD14 in turn has been associated with IFN-γ and IL-4 cytokine responses (6), while CD14 -550T has been associated with higher sCD14 in breast milk and lower sCD14 in plasma (11). We however found no associations between these variants and sCD14 levels. Our results do agree with Guerra et al. (11) in that we also show a significant link between sCD14 and -1619C/T. Replication of CD14 genetic studies has proved
controversial and likely depends on a number of factors including environmental exposures and ethnicity (14). CD14 -159 TT homozygotes have been shown to have higher anti-pneumococcal vaccine antibody levels in children (13). We however did not find any associations with CD14 genetic variants and measles vaccine IgG responses. Therefore, we suggest that alternative immunogenetic factors are likely to have stronger effects on measles-specific antibody responses, and thus the occurrence of measles vaccine failure.

Genetic association studies with measles vaccine responses have predominantly been in Caucasian populations, a strength of our study is that we are the first to evaluate genetic determinants of measles responses in children from a developing country where measles is endemic. We were therefore able to include actual cases of vaccine failure (compared with just low seronegative antibody levels like in our Australian cohort). However, small numbers of cases necessitate that our findings be replicated in future studies. Our study also emphasizes the difficulty in diagnosing measles cases, with clinical (symptoms only) and laboratory (symptoms and IgM confirmation) cases definitions yielding different results.

Determining the factors that drive inadequate immune responses and the development of vaccine failure are particularly important in developing countries, where measles is endemic and children suffer more severe disease and death. Knowledge in this area may inform as to which children are likely to be at high risk, and this may lead to the initiation of more effective vaccine strategies for these children. Elucidating all of the contributing immunogenetic factors is also important for the future of vaccine research as we aim towards a more targeted, personalised approach to vaccinology (15), as well as moving toward the ultimate goal of worldwide measles elimination.
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