Antibody Kinetics in Infants Exposed to Chikungunya Virus Infection During Pregnancy Reveals Absence of Congenital Infection

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To search for serological evidence of congenital infection in apparently healthy neonates born to women infected with the Chikungunya virus (CHIKV) during pregnancy, monitoring for CHIKV-specific antibodies was performed within the CHIMERE cohort study (Reunion island, 2006–2008). CHIKV-specific antibody kinetics showed no evidence of asymptomatic congenital infection as neonates were tested negative for CHIKV-specific IgM antibodies at birth and 368 infants with CHIKV-specific IgG antibodies seroreversed completely (mean seroreversion time: 7.7 months). Seroreversion time of transplacental CHIKV IgG antibodies was inversely correlated with the stage of pregnancy at which exposure took place and end-term small for gestational infants seroreversed earlier.

Keywords. Chikungunya virus; pregnancy; congenital infection; transplacental transfer; serum immunoglobulins G; serology; immunization; neonate; cohort study; Kaplan-Meier.

The Chikungunya virus (CHIKV), a re-emerging alphavirus transmitted by *Aedes* mosquitoes, has become a global concern in recent years, causing major epidemics and imported cases worldwide [1]. CHIKV infection is most often mild, characterized by fever, rash and arthralgias but can sometimes give rise to severe pathology, especially in neonates and the elderly [2]. Biological diagnosis is by reverse transcriptase polymerase chain reaction (RT-PCR), positive during the first week of illness, or to CHIKV-specific capture ELISA Immunoglobulin M antibodies (IgM abs), a positive result being obtained on average 5–7 days after onset of symptoms [3]. For epidemiological purposes, lifelong CHIKV-specific ELISA immunoglobulin G (IgG) abs are used to establish infection in previously apparently healthy subjects [4]. Importantly, the switch from IgM to IgG abs might be delayed, and CHIKV-specific IgM abs remain in the host for many years and are indicative of long-term persistence of CHIKV antigens [5, 6].

During the 2005/2006 epidemic, cases of intrauterine death and life-threatening perinatal mother-to-child CHIKV transmission were reported in La Réunion, India and Sri Lanka [6–11], raising the possibility of undiagnosed congenital infection. Most pregnant women recovered completely from CHIKV infection and gave birth to apparently healthy infants, testing negative for CHIKV IgM but sometimes positive for IgG abs [8, 9]. However, screening for specific IgM abs in neonatal or cord blood may be unreliable, and it is possible that *in utero* production of CHIKV-specific abs is impaired and/or insufficient to be detected in congenitally infected infants as described in some early fetal infections [12]. Serial measurements of pathogen-specific IgG abs are usually used to check for increasing or persistent levels which may indicate an immune response in the congenitally-infected infant or rule out congenital infection when the presence of transplacental IgG abs subsides.

The objectives of our multicenter prospective study were (a) to determine whether apparently healthy neonates exposed to CHIKV during pregnancy presented serological evidence of asymptomatic congenital CHIKV infection by long-term monitoring of CHIKV-specific abs in their peripheral sera and (b) to assess factors associated with the persistence of CHIKV-specific IgG abs in children.

POPULATION AND METHODS

The CHIMERE (Chikungunya Mère-Enfant) cohort study included 653 liveborn neonates born to 658 women CHIKV-infected during pregnancy during the 2005/2006 epidemic in La Réunion. Serological status for CHIKV infection was obtained on inclusion of the mother in the study, during pregnancy. Subsequent reports of maternal chikungunya infection were confirmed using serological testing or RT-PCR. Serological tests

with negative results were repeated after birth of the infant. Phase of pregnancy at point of infection was determined by checking patient history of symptoms or using RT-PCR when available [9]. Sera were collected for IgG and IgM class abs against CHIKV-specific antigens from infants during the neonatal period (day 3 and week 3 following birth) to detect early or late-onset CHIKV infection. Seropositive asymptomatic infants with CHIKV-specific IgG abs were clinically monitored using a standardized protocol with serological assessment at 3, 6, 9, 12, 18 and 24 months of age, unless seroreversion (seronegativation) took place. Episodes of chikungunya-like infection were recorded. CHIKV-specific immunoglobulins were measured using an ELISA technique automated with an EtiMax 3000 apparatus (DiaSorin, Italy). Sera were considered positive when sample optical density (OD)/negative OD was >2.0 [8]. Written informed consent was obtained from the parent/ guardian. The study was approved by the ethics committee of Tours, France (no. 2006–2007) and was reported to the French Data Protection Authority. Seroreversion curves were drawn using the Kaplan-Meier method to describe the survival time (time to seroreversion) of CHIKV-specific IgG abs in children.

The average onset of maternal infection (gestational age), the interval between infection and birth (mean, ≤14 days, >14 days), gestational age (mean, preterm vs full-term) and birth-weight (mean, small for gestational age vs normal for gestational age) were compared between children monitored in the study and those unable to be included in the follow-up using the Mann–Whitney, Chi-square or Fisher exact tests as appropriate, to allow accurate interpretations of seroreversion times, taking into account the possibility of censoring and repartition bias.

Of the 653 live-born neonates exposed to maternal infection during pregnancy, only one developed clinical CHIKV infection with positive result from RT-PCR in serum and cerebrospinal fluid on day 6 following birth. Initial screening for CHIKV-specific IgM and IgG abs was negative and IgM abs were subsequently detected in week 3 and IgG abs at one month following birth. On follow-up, high levels of CHIKV-specific IgG abs were still observed at 18 months of age. This particular neonate was born from a mother who had acute CHIKV infection symptoms on the day before delivery (positive result for maternal RT-PCR on day 2 *postpartum*) and was excluded for further analyses [9]. Of the 652 remaining asymptomatic infants, 62 were excluded because of the lack of blood sampling during the neonatal period, leaving 590 infants eligible for serologic follow-up.

RESULTS

None of the 590 newborns tested had detectable titres of IgM abs on day 3 following birth and ten neonates were negative for CHIKV-specific IgG abs. Of the seven infants born to a mother infected in the two weeks preceding delivery, IgG abs were detected for three neonates (43%), and conversely, 99% of the neonates born to mothers who developed CHIKV infection more than 15 days before delivery were seropositive. Of the 580 CHIKV-IgG seropositive infants monitored in the
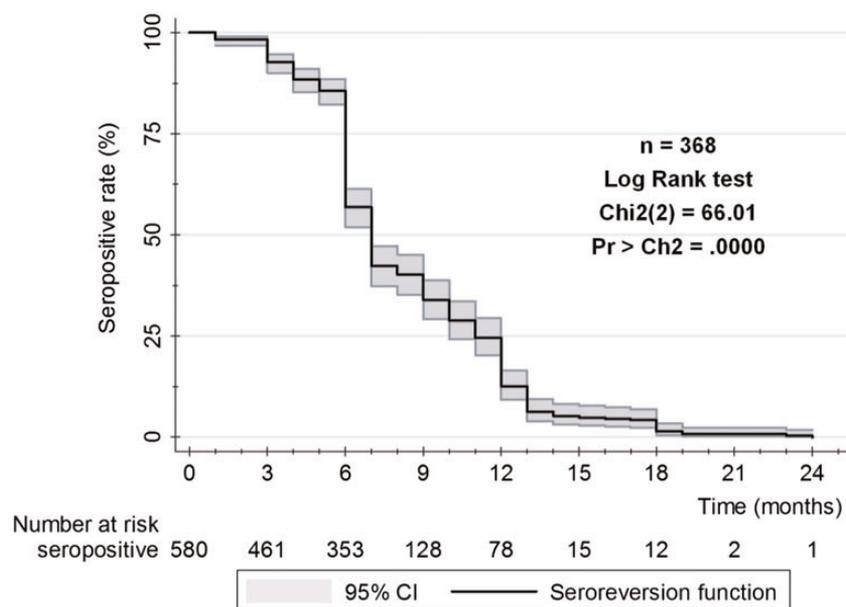

Figure 1. Overall seroreversion curve for Chikungunya virus immunoglobulin G antibodies for children exposed to maternal infection during pregnancy. Seroreversion time during the 2-year monitoring in 580 children. IgG-seropositive children at risk are given for the beginning of each month from birth date. Seroreversion was observed for 368 children.
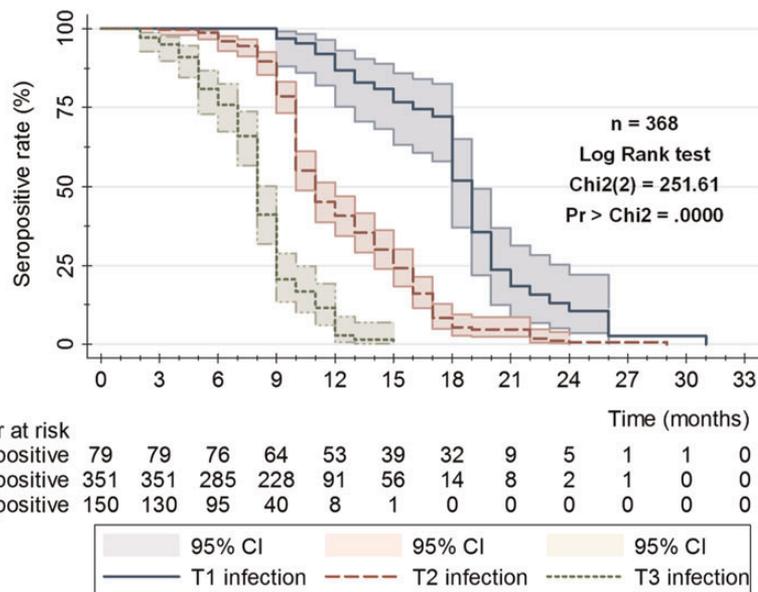
Figure 2. Serorevesion curves for Chikungunya virus immunoglobulin G antibodies for children exposed to maternal infection during pregnancy by trimester of exposure. Seroreversion time during the 2-year follow-up in 580 children: 79 with first-trimester infection (T1), 351 with second-trimester infection (T2), 150 with third-trimester infection (T3). IgG-seropositive children at risk are given for the beginning of each month and are grouped by onset of maternal infection. Seroreversion was observed for 368 children and the last seronegativation occurred 31 months after maternal infection.

study, more than 90% remained seropositive beyond the third month, >50% beyond the sixth month, >30% beyond the ninth month, >10% beyond the first year, and none beyond the second year (Log-Rank test = 66.01, $P < .001$, Figure 1). Of the 368 initially CHIKV-specific IgG seropositive infants for whom complete follow-up data was available, all seroreversed (mean time to seroreversion: 7.7 months, standard deviation 3.3; last seroreversion observed at 24 months) and none subsequently developed proven CHIKV infection.

Time to seroreversion correlated with the timing of the exposure to CHIKV infection during pregnancy with more than 75% of children having persistent IgG abs more than one year after maternal infection in first-semester exposure, >30% in second semester-exposure, and less than 1% in third-semester exposure (Log-Rank test = 251.61, $P < .001$, Figure 2). This correlation was still observed when categorizing children by gestational age (Supplementary File 1, Figure 3) or birth-weight class (Spearman's rho coefficient: $\rho = -0.7355$, $P < .001$, in normal for gestational age infants; $\rho -0.7057$, $P < .001$, in small for gestational age infants). Moreover, CHIKV-specific IgG abs were detected early, as in a very premature infant born at 26 weeks and in the group of children with overt seroreversion (n = 368), the average time to seroreversion was shorter in end-term (>39 weeks) small for gestational age (SGA) infants than in their end-term (>39 weeks) normal for gestational age (NGA) counterparts (Mann–Whitney test, 6.5 vs 8.0 months, $P = .024$). Thus, of the 159 initially CHIKV-specific IgG seropositive end-term (>39 weeks) infants, 60% remained positive

beyond six months of age in the NGA group, against 40% in the SGA group (Log-Rank test = 4.05, $P = .044$, Supplementary File 2, Figure 4).

Maternal infection occurred earlier in pregnancy ($P < .001$) and the gestational age at birth was higher ($P = .031$) for infants unable to be included in the follow-up (n = 212) compared to those with complete serological follow-up (n = 368) (Supplementary File 3, Table 1), indicating that the fetus was prone to placental transfer of IgG antibodies for a longer period in the "lost to follow-up" group.

DISCUSSION

The chikungunya epidemic which occurred in an immunologically naive population in Réunion island provided a unique opportunity to study antibody kinetics in infants exposed to CHIKV during pregnancy. Our study found no evidence for congenital infection in asymptomatic live-born infants exposed to CHIKV during pregnancy, as confirmed by the absence of CHIKV-specific IgM abs during the neonatal period and the progressive decay of transplacental CHIKV-specific IgG abs in seropositive infants. It should be noted that neonates are capable of developing long-lived immune responses following CHIKV infection shortly after birth, as demonstrated by a historical cohort of children (n = 36) with perinatal mother-to-child infection: all children still tested highly positive for IgG abs at an average of 18.3 (±5.5) months following birth (personal unpublished data).

Placental transfer of CHIKV-specific IgG abs and rate of seropositivity was correlated with the timing of maternal infection with antibodies remaining longer in infants exposed to infection earlier in pregnancy. This correlation was still observed when categorizing children by gestational age or birth-weight, highlighting the absence of major influence of these variables (proxies of placental maturation and placental weight) on the time taken for seroreversion. Seropositivity persisted longer in our study than previously observed in a Thai study (seroreversion rate of 100% at 9 months of age) [13]. Infants delivered within two weeks of maternal infection tended to have undetectable levels of CHIKV-specific IgG abs and end-term SGA infants seroreversed earlier. Taken altogether, our data suggest that the duration of prenatal exposure to maternal antibodies is critical to the survival time of transplacental IgG abs throughout infancy, and that fetal (or placental) growth is also involved in late pregnancy (>39 weeks). While the impermeability of the placental barrier to CHIKV was demonstrated in vitro [14], it is hypothesized that *peripartum* cases of CHIKV mother-to-child infection occur as there is no time for protective maternal antibodies to be formed and to be passed to the neonate, who is contaminated directly through placental breaches during labour in viremic mothers [8]. There is no indication for obstetrical intervention in cases of maternal CHIKV infection during pregnancy as the fetus will benefit from the transfer of putatively protective maternal antibodies. Our findings support several proposed mechanisms of placental transfer following maternal infection in pregnancy: (1) transplacental IgG transfer begins as early as 13 weeks gestation and increases as the placenta matures (2) transport of IgG depends on maternal levels of antibodies, and (3) transfer efficacy is probably not impaired by absence of infection-induced placental injury [15]. However, the fact that there is a correlation between late maternal infection and the duration of maternal antibodies during infancy does not support the idea that antibody transfer increases in a linear fashion as pregnancy progresses, with the largest amount transferred in the third trimester. The observation of an extended period of placental IgG antibody transfer in the "lost to follow-up" group suggests a possible repartition bias (right censoring) which is likely to reduce overall seroreversion time. However, given the small average difference (two weeks gestation), it is unlikely that this bias would significantly alter the seroreversion curves.

The observation that no postnatal mosquito-borne infection was recorded during monitoring must be taken with caution. This could be explained by the putative protection of transplacental CHIKV-specific IgG abs or by the sudden decline of the epidemic at the beginning of our study [9]. However, in our pediatric experience, early infantile CHIKV mosquito-borne infections were observed in infants born to mothers without history of Chikungunya during pregnancy.

Our study provides valuable information for caregivers and for parental counselling after CHIKV infection of the mother during pregnancy. Monitoring of children with transplacental antibodies reveals no congenital infection. Seroreversion time of transplacental CHIKV-specific IgG antibodies is linked to the timing of exposure during pregnancy. These issues will be helpful for maternal or postnatal immunization, should a vaccine become available.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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