Pre-existing maternal IgG antibodies as a protective factor against congenital cytomegalovirus infection: A mother-child prospective cohort study

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Summary

Background An understanding of the correlation between maternal immunity and congenital cytomegalovirus (CMV) infection is critical for informing the design and evaluation of an effective maternal vaccine. This study aimed to quantitatively measure the protective effect of pre-existing maternal immunity against congenital CMV (cCMV) infection.

Methods A mother-child cohort study was conducted in three maternal and child health hospitals in China from 2015 to 2018. Pregnant women were consecutively enrolled, and anti-CMV pp150 IgG concentration at early, middle and late gestational ages were evaluated. Their newborns were screened for cCMV infection by CMV-DNA testing of saliva and urine.

Findings In total, 6729 pregnant women were enrolled, and 6602 of them (98·11%) were positive for CMV IgG at their early gestational age visit (median time: 13 gestational weeks (GW); time range: 6–25 GW). In total, 6228 live newborns were born to seropositive mothers, and 48 (0·77%) of these infants were diagnosed with cCMV infection. The geometric mean concentration (GMC) of CMV IgG at an early gestational age in the women who delivered cCMV-positive newborns (i.e., the transmitters) was 8·54 IU/mL; this was significantly lower than the GMC in the non-transmitters (11·01 IU/mL; P=0·04). In early gestation, the risk of cCMV infection decreased as maternal IgG antibody levels increased (P=0·020); however, the same was not true in middle or late gestation (P>0·05). Using receiver operating characteristic analysis, a CMV IgG concentration of 12·83 IU/mL was established as the optimal diagnostic threshold. Compared to lower levels of CMV IgG (<12·83 IU/mL) in seropositive pregnant women, higher maternal CMV IgG levels (≥12·83 IU/mL) were associated with a 50% reduction in cCMV infection risk in infants (relative risk=0·50; 95% confidence interval: 0·27–0·93; P=0·028).

Interpretation For seropositive women, a higher level of CMV IgG at an early gestational age is associated with a lower risk of cCMV infection in their newborns.

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Introduction

Cytomegalovirus (CMV) infection is extremely common, and over half of adults worldwide are infected.[1,2] Following primary infection, CMV establishes a lifelong latent infection within the host, with periodic

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Research in context

Evidence before this study

We searched PubMed, Embase and Web of Science for studies published between database inception and Oct 11, 2021, using the search terms ((maternal OR pregnan* OR mother) AND (immun* OR antibod* OR IgG) AND (cytomegalovirus OR CMV) AND (congenital OR foetal OR vertical transmission OR Intrauterine OR Prenatal) AND infection AND (protect* OR efficacy OR effect* OR against OR prevent*)), without language restrictions. Robust and straightforward evidence is lacking on the specific contribution of maternal immunity in preventing intrauterine infection in immunocompetent seropositive women. By comparing the prevalence of cCMV infection in newborns born to seronegative mothers and seropositive mothers, several studies have indicated that natural acquired CMV immunity in mothers is partly but not entirely effective against intrauterine transmission. In contrast, several studies reported that maternal immunity had no protective effect on infants, as the mothers of cCMV-positive infants did not have significantly lower IgG titres than the mothers of uninfected infants. These studies had limited statistical power because the numbers of newborns with cCMV were very small, and in some of them, the maternal antibody status was tested near the time of delivery or an uncertain period before pregnancy. Existing evidence, mainly from primary infection in seronegative populations, may provide a basis for the notion that immunity can prevent exogenous CMV infection; however, the protective effect of immunity against endogenous infection remains unclear given the challenge of distinguishing between exogenous and endogenous CMV infection. The unclear protective effect of maternal immunity is one of the major impediments to developing a vaccine for preventing cCMV infection in newborns born to seropositive women.

Added value of this study

In this large-scale mother-child cohort study, the quantifiable relationship between CMV IgG level in early gestation (approximately 13 GW) and the outcome of cCMV infection in newborns of immunocompetent seropositive pregnant women was analysed. The risk of cCMV infection decreased as maternal anti-CMV pp150 IgG antibody levels increased in early gestation. In the population with an IgG higher than 12·83 IU/mL, which was close to the median level in the population, the risk of intrauterine transmission was half as high as in the population with an IgG lower than 12·83 IU/mL. This evidence demonstrates that pre-existing anti-CMV humoral immunity could partly protect against intrauterine infection of offspring in immunocompetent seropositive women.

Implications of all the available evidence

Increased CMV-specific IgG in immunocompetent seropositive women is associated with a reduced risk of intrauterine CMV infection of their offspring, which suggests that seropositive women may benefit from CMV vaccination.

reactivation[3,4]; additionally, a patient can be reinfected with a different CMV strain.[5] In the general population, the specific clinical symptoms caused by primary CMV infection are rarely noticed; however, in congenitally infected children and immunosuppressed individuals, CMV infection can lead to severe adverse clinical outcomes. Congenital CMV (cCMV) infection is the most common nongenetic cause of disabilities and birth defects globally.[6] Both primary infection in seronegative pregnant women and nonprimary infection (reactivation or reinfection) in seropositive pregnant women can cause intrauterine CMV infection of foetuses; the latter causes the majority of cCMV cases globally.[7]

The development of a CMV vaccine is considered a priority by the international scientific community. Pregnant women or women of childbearing age (including CMV-seronegative and CMV-seropositive women) are thought to be potential target populations for CMV vaccination against cCMV infection.[8] Several preventive CMV vaccine candidates have been tested in clinical trials, but none of them has been licenced.[9] An efficacy of approximately 50% against primary infection in seronegative pregnant women was attained in a phase II clinical trial of a recombinant subunit vaccine.[10] However, the specific contribution of immunity against nonprimary infection (reactivation and reinfection) in seropositive women has not been clearly demonstrated, which is partly due to the notable challenges in identifying nonprimary infection due to the uncertainty of serological and virological changes following nonprimary infection.[3,4,11]

In a recent large-scale mother-child prospective cohort in China, we demonstrated that the majority (98·0%) of cCMV infections among newborns came from seropositive mothers. In this report, we aimed to understand the change in maternal immunoglobulin (IgG) antibody levels during pregnancy in seropositive women and quantitatively measured the protective effect of maternal natural acquired immunity against cCMV infection in this cohort.

Methods

Study design

From June 2015 to September 2017, we recruited pregnant women at their first antenatal visits, with follow-up during middle and late gestation, at three county-level maternal and child health hospitals (MCHHs), located in Xinmi, Zhongmu and Jiaxian, Henan Province, China. Serum samples were collected at each visit

during pregnancy, and the CMV IgG antibody level was quantified.

Infants born to the participants were screened for cCMV infection by testing saliva and urine samples within 13 days after birth. Confirmation testing was performed using subsequent saliva and urine samples, typically collected typically within 21 days of birth.[12] The sociodemographic information of the pregnant women and newborns was collected on enrolment and on delivery.

Specimen collection

Serum was obtained from maternal blood for serological testing. A sterile cotton swab was used to collect newborn saliva at least one hour after feeding; the sample was placed in transport medium (DMEM) immediately after collection. Urine specimens were collected using infant urine drainage bags (GuanKe Bio, Ningbo, China), and faecal contamination was carefully avoided.

Laboratory testing

Serum samples were tested for IgG antibodies against CMV using a well-validated enzyme-linked immunosorbent assay based on the pp150 antigen, as previously reported.[13] The IgG antibody levels were quantitated according to the standard curve of the Paul Ehrlich Institute (PEI) reference preparation of CMV IgG (Paul-Ehrlich-Institut, Germany). Briefly, 96-well microplates were coated with purified pp150 (UL32) protein at 1·0 μg/mL at 4°C overnight. After a blocking step, 100 μL of 100-fold diluted serum was added to each well and incubated at 37°C for 1 h. After 5 washes, horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) 3000-fold diluted was added and incubated for 30 min at 37°C, and TMB was added for colour development after another washing step. The optical density at 450 nm (OD_{450}) was read by a microplate spectrophotometer (Autobio, Zhengzhou, China). For quantification of the anti-CMV IgG antibodies, a standard curve was included in each microplate, using the PEI reference preparation of CMV IgG as the standard. The antibody level of each sample was calculated from the corresponding standard curve. The linear range was 0·042–0·31 U/mL. The samples with an OD value above the linear range were further diluted. For samples with an OD value lower than the lower range, 10-fold diluted samples were assessed, and the antibody levels were calculated.

The saliva and urine samples from the newborns were tested for CMV-DNA by real-time PCR, as reported previously.[12]

To prevent inter-laboratory and inter-run variance, all serum samples and all newborns' samples were tested in one laboratory (National Institute of Diagnostics and Vaccine Development in Infectious Diseases) by the same staff, and all samples from the same mother were specifically organized together and tested in the same run.

Definition of cCMV infection

cCMV infection (or intrauterine infection) was defined as being present if a newborn with a positive result from CMV-DNA PCR in saliva and/or urine within 13 days of birth was further confirmed to be positive in a subsequent saliva and/or urine test within 21 days of birth.[12] Women who delivered at least one cCMV newborn in this pregnancy were defined as transmitters; women with uninfected newborns were defined as non-transmitters. For twin/multiple pregnancies, the woman was defined as a transmitter if any of the newborns was diagnosed with cCMV infection.

Statistical analysis

The geometric mean concentration (GMC) and 95% confidence interval (CI) were used to describe the mean IgG antibody concentration. The raw IgG data did not follow the normal distribution; hence, further analysis of covariance and trend analysis were based on $\log_2$-transformed IgG (logarithmic transformation is a widely used method to adjust IgG data to follow the normal distribution). Seropositive pregnant women with completed serological testing during early/middle/late gestation were included for analysis of the antibody change during pregnancy. Analysis of covariance was performed by regressing GW and maternal age to test the difference in IgG levels between transmitters and non-transmitters. Polynomial-based trend analysis of repeated measures was conducted to understand the trend of IgG change with pregnancy and to test the difference in trend between transmitters and non-transmitters. In each individual, the IgG ratio was calculated by dividing IgG in late gestation by IgG in early gestation, which reflects the fold change in IgG between these stages; the median value and IQR of the IgG ratio in transmitters and non-transmitters are reported. Additionally, the differences in the rates of twofold/fourfold increases in IgG from early to late gestation in transmitters/non-transmitters were analysed in a logistic regression analysis that adjusted for the interval of GW between the early and late gestational sampling times.

Pregnant women with serological testing at the first pregnancy visit and confirmed diagnostic results of newborn cCMV infection were included in the analysis of the relationship between the baseline antibody level and cCMV infection. The 2-sided 95% CIs for the estimates of the prevalence of cCMV infection were calculated on the assumption of a Poisson distribution. Four groups were defined according to quartiles of IgG concentration in early gestation to calculate the prevalence

of intrauterine infection under different IgG levels. The Cochran–Armitage trend test was applied to analyse the trend in the prevalence of intrauterine infection with rising maternal IgG antibody levels. Receiver operating characteristic (ROC) analysis was employed to evaluate the diagnostic ability of the binary classifier as a function of the discrimination threshold. The threshold of maternal IgG for classifying low and high cCMV risk groups was defined under the highest Youden value (sensitivity + specificity - 1) when predicting cCMV infection. To assess the association between maternal antibody levels and cCMV infection, the relative risk (RR) and 95% CI were estimated by Poisson regression models (GENMOD procedure). Furthermore, the adjusted RR was estimated by adjusting for maternal age and GW at an early gestational age using a multivariate model. All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC).

Ethics

The study was approved by the Ethical Committee of the School of Public Health, Xiamen University (ClinicalTrials.gov, NCT02645396). Informed consent was obtained from each participating mother at enrolment. The study was granted by the National Natural Science Foundation of China, Merck Sharp & Dohme Corp., and Xiamen University. A total of 22 references were referred in the study.

Role of funders

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Results

Study population

A total of 6729 pregnant women were enrolled and underwent CMV serological testing in the early phase of pregnancy (median time: 13 gestational weeks (GW), time range: 6–25 GW), and 6602 (98·11%) were CMV seropositive (Fig. 1). Among the seropositive women, 5537 pregnant women completed CMV serological testing at early, middle and late gestational ages (median time (time range) was 13 (6–22), 25 (23–34) GW and 38 (34–42) GW, respectively), and 6228 newborns were followed up and underwent cCMV screening, with 48 (0·77%) defined as having cCMV infection. A total of 6121 pregnant women underwent IgG testing in early gestation and had newborns with a confirmed diagnosis of cCMV infection; these mothers' sociodemographic information is shown in Table 1. The detailed screening results of the newborns with cCMV have been reported in our previous study.[12]

Antibody change during pregnancy in seropositive women

A total of 5537 pregnant women underwent a series of serological tests during pregnancy, and 5284 of those women were successfully followed up until delivery. The GMCs of anti-CMV pp150 IgG in 5537 women during early (Fig. 2a), middle and late gestation were 10·97 IU/mL (95% CI: 10·73–11·21 IU/mL), 11·97 IU/mL (95% CI: 11·70–12·23 IU/mL) and 12·21 IU/mL (95% CI: 11·95–12·49 IU/mL), respectively. The IgG concentration tended to increase with GW (F=23·56, P<0·0001), and the increase was greater in transmitters (N=43) than in non-transmitters (N=5241) (F=9·33, P=0·002) by polynomial-based trend analysis, as shown in Fig. 2b. In early gestation, the GMC of IgG was significantly lower in transmitters (8·54 IU/mL; 95% CI: 6·51–11·19) than in non-transmitters (11·01 IU/mL; 95% CI: 10·77–11·26 IU/mL; P=0·034) by analysis of covariance; in middle and late gestation, however, the difference was nonsignificant (P=0·15 and 0·73, respectively; Fig. 2b).

In each individual, the IgG ratio was calculated by dividing IgG in late gestation by IgG in early gestation, which reflects the fold change in IgG from early to late gestation. The median IgG ratio was 1·13 (IQR: 1·00–1·63) in transmitters and 1·08 (IQR: 0·89–1·35) in non-transmitters. Overall, 76·74% (33/43) of transmitters had an IgG ratio greater than 1, which means that the IgG concentration was higher in late gestation than in early gestation; the percentage was lower in non-transmitters (3162/5241, 60·33%, P=0·040, multivariate logistic analysis) (Fig. 2c). A fourfold increase in IgG (IgG ratio $\geq$ 4) was observed in 6·98% (3/43) of transmitters and 1·39% (73/5241) of non-transmitters (P=0·0077, multivariate logistic analysis), and a twofold increase in IgG (IgG ratio $\geq$ 2) was observed in 16·28% (7/43) of transmitters and 7·86% (412/5241) of non-transmitters (P=0·047, multivariate logistic analysis).

Through comparison of sociodemographic information in all seropositive pregnant women and seropositive pregnant women with a series of serological tests (Supplementary material 1), only a very minor difference was shown in residence, which was not a variable significantly related to intrauterine infection. As a sensitivity analysis, we also performed the above analysis in all seropositive pregnant women, and the results remained similar (Supplementary material 2). The GW of sample collection in early, middle and late gestation and the GW interval between early and late gestation

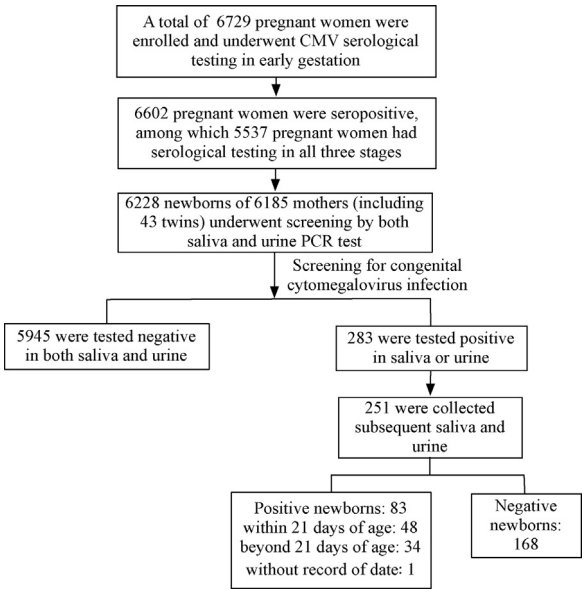

Figure 1. Flow diagram of participant enrolment and follow-up.

were comparable between the transmitters and non-transmitters (Supplementary material 3).

The risk of cCMV infection is associated with the pre-existing maternal IgG antibody level

A total of 6121 pregnant women underwent IgG testing at an early gestational age received a confirmed diagnosis of newborn cCMV infection; their data were included in the data set for this part of the study. Four groups were defined according to quartiles of IgG concentration in early gestation (0·42–6·55, 6·55–12·23, 12·23–19·94, 19·94 and above, IU/mL), and the prevalence rates of cCMV in the four groups were 1·05% (18/1549, 95% CI: 0·69–1·83), 0·86% (13/1511, 95% CI: 0·46–1·47), 0·72% (11/1531, 95% CI: 0·36–1·28) and 0·39 (6/1530, 95% CI: 0·08–0·85), respectively; the risk of intrauterine infection decreased with increasing maternal IgG antibody levels in early gestation as calculated by the Cochran–Armitage trend test (Z=-2·33, P=0·020) (Fig. 2d).

According to the ROC analysis, the optimal cut-off value for the best differentiation of the low- and high-risk groups was 12·83 IU/mL, under which the Youden value was highest (0·18). Fifteen (0·51%) of the 2915 women whose IgG levels were higher than 12·8 IU/mL delivered newborns with cCMV infection, and the rate was half of that in women whose IgG levels were lower than 12·83 IU/mL (1·06%, 34/3206; RR=0·45, 95% CI: 0·24–0·87, P=0·012, Poisson regression analysis). As maternal age differed significantly between the transmitter and non-transmitter groups (Table 1) and GW of sampling was related to IgG levels, the RR was further adjusted for maternal age and GW of sampling at the early gestational age (adjusted RR: 0·50, 95% CI: 0·27–0·93, P=0·028, multivariate Poisson regression analysis) (Table 2).

	TransmittersNo (%)	Non-transmitters*No (%)	P_1 value#	P_2 value#
Overall	48 (0·85)	5599 (99·15)	-	
Hospital				
Xinmi MCHH	31 (0·88)	3494 (99·12)	0·90	
Jiaxian MCHH	12 (0·85)	1400 (99·15)		
Zhongmu MCHH	5 (0·70)	705 (99·30)		
Maternal age in years Mean (SD)	24·6 (3·4)	26·9 (4·3)	0·04	<0·001
Residence*				
Rural area	29 (0·86)	3334 (99·14)	0·90	
Urban area	19 (0·83)	2265 (99·17)		
First pregnancy*				
Yes	27 (1·05)	2534 (98·95)	0·13	
No	21 (0·68)	3065 (99·32)		
Primiparous*				
Yes	38 (1·05)	3577 (98·95)	0·03	0·21
No	10 (0·49)	2022 (99·51)		
Singleton pregnancy				
Yes	47 (0·84)	5562 (99·16)	0·24	
No	1 (2·63)	37 (97·37)		

***Table 1*: Maternal sociodemographic information.**

* Note: 474 non-transmitters who did not have complete sociodemographic information were excluded from the analysis. #P_1 is the P value from univariate analysis; P_2 is the P value from multivariate analysis including the variables of maternal age and primiparous status.

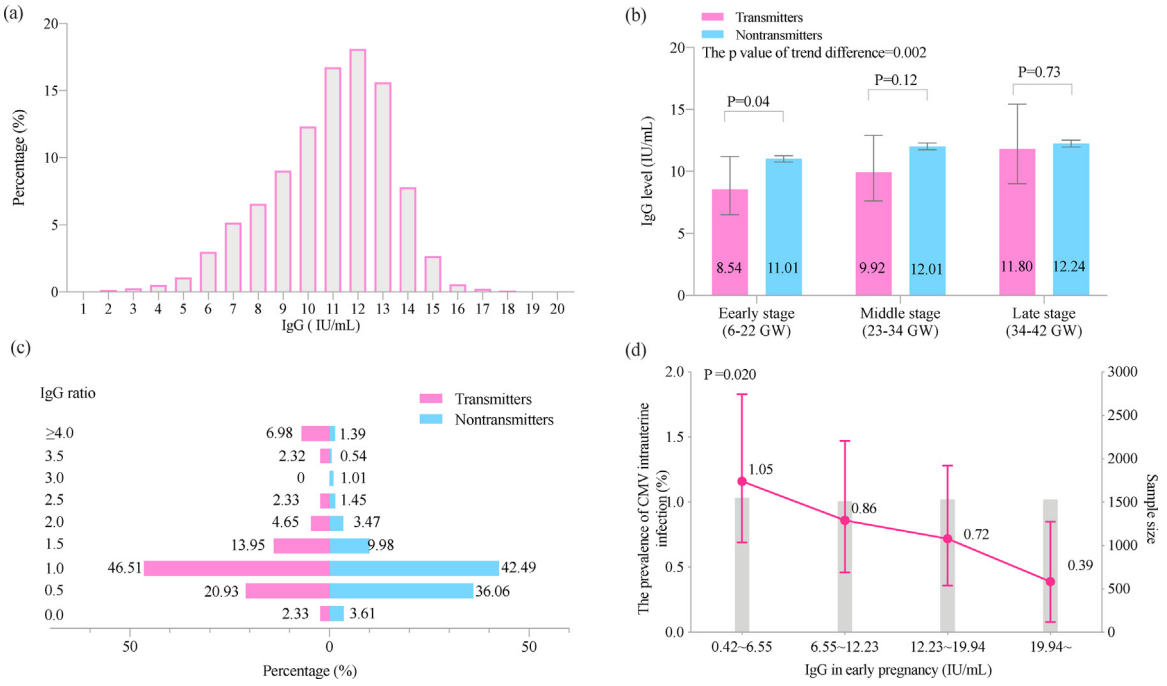

Figure 2. Maternal IgG during pregnancy and its relationship with intrauterine infection. (a) The distribution of IgG concentration in early gestation in seropositive women. All 6602 pregnant women who underwent IgG testing at early gestational age were included in the analysis. (b) IgG level during pregnancy in CMV transmitters and non-transmitters. Note: The sample sizes of transmitters and non-transmitters were 43 and 5241, respectively. The bar at each point indicates the 95% CI of IgG GMC. The median and range GW of these women in early, middle and late gestation were 13 (6—22) GW, 25 (23—34) GW and 38 (34—42) GW, respectively. (c) The frequency distribution of IgG ratio in transmitters and non-transmitters. The IgG ratio was calculated by dividing IgG in late gestation by IgG in early gestation to reflect the fold change in IgG from early to late gestation in each individual. The sample sizes of transmitters and non-transmitters were 43 and 5241, respectively. (d) The prevalence of intrauterine CMV infection in women with different IgG antibody levels in early gestation. The bar at each point denotes the 95% CI. The grey column represents the sample size. Four groups were defined according to quartiles of IgG concentration in early gestation. The sample sizes in the four groups were 1549, 1511, 1531 and 1530. The *P* value was calculated by the Cochran—Armitage trend test.

Discussion

This study quantitatively analysed the relationship between CMV IgG concentration in early gestation (approximately 13 GW) and cCMV infection in newborns of immunocompetent seropositive pregnant women. The GMC of IgG in early gestation in seropositive women was 10·97 IU/mL (95% CI: 10·73—11·21 IU/mL). The risk of cCMV infection decreased in a reverse manner with maternal IgG antibody levels in early gestation. A maternal IgG level that was higher than 12·83 IU/mL in early gestation decreased the risk of cCMV infection in their newborns by approximately half (RR= 0·50, 95% CI: 0·27—0·93) compared with women with IgG antibodies lower than 12·83 IU/mL.

It is known that seronegative women will benefit from vaccination, as a phase 2 clinical trial revealed that vaccination had an efficacy of approximately 50% against primary infection.[14] A study by Fowler et al.

	Maternal level of IgG (IU/mL)	No. of pregnant women delivering a live neonate	No. of pregnant women delivering a neonate with cCMV infection (%)	RR (95% CI), *P*	Adjusted RR (95% CI), P*
Early pregnancy (6-22 GW)	<12·83	3206	34 (1·06)	0·45 (0·24, 0·87), 0·012	0·50 (0·27, 0·93), 0·028
	≥12·83	2915	14 (0·48)		
	Total	6121	48 (0·78)		

***Table 2*: The association of maternal IgG antibody in early pregnancy with the outcome of cCMV infection in newborns.**

* Note: The adjusted RR was estimated by adjusting for maternal age and GW at an early gestational age. Women with IgG concentration less than 12·83 IU/mL constituted the reference group. GW: gestational weeks. The IgG GMCs of women with IgG concentration lower and higher than 12·83 IU/mL, respectively were 5·87 (5·75, 6·00) and 21·62 (21·34, 21·90), respectively.

comparing the prevalence of cCMV infection in newborns born to seronegative mothers and seropositive mothers showed that natural acquired immunity resulted in a 69% reduction in the risk of cCMV infection.[15] This finding demonstrated the protective effect of natural acquired immunity, although the effect was underestimated because the serostatus was determined by testing cord serum from the previous delivery, and seroconversion could occur between two pregnancies; meanwhile, viral exposure in seropositive women (both exogenous and endogenous CMV infection, i.e., reinfection and reactivation) was more frequent than that in seronegative women (exogenous CMV infection). Existing evidence mainly from primary infection in seronegative populations may be a basis for the notion that immunity is capable of preventing exogenous CMV infection; however, the protection of immunity against endogenous infection remains unclear given the challenge in distinguishing exogenous and endogenous CMV infection. Hence, in seropositive women, sustained natural acquired CMV immunity is speculated to be partly but not entirely effective against future CMV infection and further intrauterine transmission. However, there is a lack of robust and straightforward evidence regarading the specific contribution of maternal immunity against intrauterine infection in immunocompetent seropositive women, which is one of the major impediments to developing a vaccine for preventing cCMV infection in newborns born to seropositive women. In this study, protection of maternal immunity against CMV intrauterine transmission in seropositive women was found, and this is the first study to reveal the quantifiable relationship between maternal preexisting CMV IgG level and the outcome of cCMV infection in newborns of immunocompetent seropositive pregnant women. With an IgG level higher than 12·83 IU/mL, which was close to the median value in the population, the risk of intrauterine transmission would be decreased by half. This makes the contribution of maternal immunity in seropositive women against intrauterine infection clearer and confirms that seropositive women can also benefit from immunogenic CMV vaccination.

A lack of protection by maternal natural acquired immunity has been reported in several previous studies, which reported that maternal IgG titres in early gestation were not significantly different between the cCMV group and uninfected group. However, in these studies, statistical power was limited since the numbers of cCMV newborns were very small (not more than 14).[16–18] A recently published study reported higher levels of antibodies in transmitters than in non-transmitters among seropositive mothers[19]; however, it was noticed that in this study, maternal serological testing was conducted using samples collected around the time of delivery when immunity was stimulated by nonprimary infection. A mild increasing tendency of IgG antibodies during pregnancy was observed in our study, and the increment was greater in transmitters than in non-transmitters. Hence, the results showed a significantly lower IgG antibody concentration in early gestation in transmitters than in non-transmitters; however, the antibody gap decreased to a nonsignificant level in middle and late gestation in our study. Thus, the period in which immunity indicators are tested may influence the apparent relationship between maternal immunity and intrauterine infection, owing to the interaction between immunity and virus activation after infection, and it is vital to conduct serological testing in early gestation or prior pregnancy. In this study, the first serological testing was performed at 6-25 GW (with a median of 13 GW), and it is expected that a greater difference might be detected between transmitters and non-transmitters before pregnancy or at an earlier gestational age.

A fourfold increase in IgG was thought to indicate an effective anamnestic antibody response, and it has been used in studies to represent nonprimary infection in seropositive pregnant women[1,20]; a twofold rise in IgG had also been applied to indicate nonprimary infection.[21] However, in this study, although the proportions of IgG with a fourfold or twofold rise from early to late gestation were greater in transmitters than in non-transmitters, in most transmitters, the IgG increment was mild (1·00- to 1·74-fold), and some transmitters displayed a decrease in antibody concentration. Both the sensitivity and the positive predictive value of a fourfold (6·4% and 3·6%) or twofold increase in IgG (17·0% and 1·7%) for predicting cCMV infection, were very low. The association of a specific antibody increase with reinfection and reactivation is currently unclear; however, in this study, the directional analysis of the relationship between maternal antibody rises during pregnancy and cCMV infection in newborns revealed that specific antibody increases would be inaccurate indicators of predicting cCMV infection.

One of the main limitations in this study is that some pregnant women entered the study relatively late in gestation (the median time was 13 GW, and the time range was 6–25 GW) owing to the generally late first antenatal visit to the hospital among Chinese women, which makes it very challenging to enroll women in very early stages of gestation. The decreasing gap between CMV transmitters and non-transmitters during pregnancy reflect the possibility that the gap before pregnancy might be even greater than our observation at earlier gestational ages; hence, the reported protective effect of maternal IgG antibodies against cCMV infection might be underestimated herein. Neutralizing antibody and cellular immunity detection were not performed in this study, owing to the great challenge of conducting labour-intensive detection in a large-scale epidemiology study. CMV-specific antibodies have been shown to effectively block viral replication in

endothelial/epithelial cells and correlate with innate immune effector functions and the magnitude of CMV-specific T cell immune responses,[22] and sustained antibodies can confer long-term immunity. In this study, we were not inferring that anti-pp150 IgG responses specifically were mediating protection against cCMV transmission, but rather measuring anti-CMV pp150 IgG antibodies as a feasible and reliable surrogate indicator of immunity induced by natural CMV infection, which have been validated to be highly correlated with antibodies against virus lysate antigens ($r = 0.865$).[13]

Although a large number of pregnant women were enrolled in this study, the number of newborns with cCMV was small due to the low prevalence of cCMV, which impacted the precision of the results. Pregnant women who met the enrolment criteria were consecutively enrolled at the participating maternal and child health hospitals, which accounted for the majority of deliveries in the three cities. The population in this study is representative of pregnant women in the region. In the multivariate analysis of the association between maternal IgG level and cCMV infection, maternal age and the GW of maternal sample collection were adjusted for; however, the potential impact of undiscovered confounding factors could not be totally excluded.

IgG reactivity at early gestation was used to classify maternal infection, which led to another concern about the misclassification of pregnant women with seroconversion from primary infection in very early gestation. Of the 48 transmitters, only one tested positive for IgM during pregnancy; that individual was IgM-negative at 15 GW but tested positive during middle and late gestation (24 GW and 38 GW), with high IgG avidity (0·81) at 15 GW (data not shown). Hence, primary infection was not observed in these transmitters. In addition, seroprevalence is extremely high (close to 100%) among childbearing females in China.[4] Most Chinese individuals are infected at a young age. The CMV seroprevalence was 97·5% in the population aged 0–14 years and increased to 98·4% in the population aged 15–59 years,[1] which was close to the seroprevalence (98·1%) observed in our study population aged 16–48 years. Therefore, we believe the number of pregnant women with primary infection and seroconversion at early gestational age will be very limited. Such a limited number of possible misclassifications would be unlikely to change the main results in this large-sample study.

In conclusion, this study revealed an association between maternal IgG antibodies and cCMV prevalence. For seropositive women, a higher level of CMV IgG in early gestation is associated with a lower risk of cCMV infection in newborns, which suggests that seropositive women may benefit from CMV vaccination.

Contributors

YH, HY, QS, CL (Caihong Li), JW, CL (Caihong Liang), SL (Shulian Li), SL (Shaowei Li), ZL, and SZ conducted the study. JT, HW, XG, QC and GZ performed the sample detection. NX, JZ, SG, TW, TL, and YS provided critical guidance during the study. YH performed the data analysis and drafted the manuscript. YH and JT verified the underlying data. JZ, SG, NX, TL, TW and YS commented on and revised the manuscript. The corresponding authors attest that all listed authors meet the authorship criteria and that no others meeting the criteria have been omitted. JZ, SG and TF are the guarantors.

Declaration of Competing Interest

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Data sharing statement

The data of this study are available from the corresponding author (Jun Zhang, zhangj@xmu.edu.cn) upon reasonable request for academic purposes.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103885.

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