Latent cytomegalovirus is associated with reduced functional antibody induction following *Plasmodium falciparum* infection

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Abstract
Latent cytomegalovirus (CMV) infection has widely reported immunomodulatory effects on the immune system, leading to altered responses to vaccination or infection with other pathogens. However, the impact of CMV on the immune response to the malaria parasite *Plasmodium falciparum* is unknown. Here we assessed antibody and cellular responses in malaria-naive volunteers during Controlled Human Malaria Infection (CHMI) with blood stage *P. falciparum* parasites. Latent CMV infection was associated with reduced induction of parasite specific IgG, IgG1, and functional antibodies following infection. Within the T-follicular helper (Tfh) cell compartment, CMV was associated with a skew towards Tfh1 cell subsets before and after malaria, and reduced activation of Tfh2 cells which have been previously associated with antibody induction during malaria. Taken together, CMV latent infection impacts the phenotype of Tfh cells required for robust antibody induction to malaria and may have particular importance in malaria endemic countries where CMV infection is almost universal and acquired early in life.

Running title
Latent CMV infection negatively impacts antibody development to malaria

Introduction

Cytomegalovirus (CMV) is a ubiquitous beta-herpes virus with a global seroprevalence estimated at 83% (Zuhair et al., 2019). CMV seropositivity ranges from less than 50% in high income countries to up to 100% in low- and middle-income countries (LMIC) (Zuhair et al., 2019), where the majority of infants can be infected in the first year of life (Kaye et al., 2008). Following infection, the virus establishes a life-long persistence, resulting in major remodelling of the immune response (Chidrawar et al., 2009). Indeed, in monozygotic twins with discordant CMV infection, over 50% of immune parameters tested were influenced by CMV infection status (Brodin et al., 2015), with impacts across the immune landscape (reviewed in (Picarda and Benedict, 2018)). This immune remodelling has consequences for subsequent pathogenesis of a broad range of diseases and induction of antibody reactivity to other pathogens (reviewed in (Moseley et al., 2023)).

The impact of CMV infection on antibody induction has been most extensively investigated with influenza vaccination in adults. However, findings are mixed, with some studies reporting a negative impact of CMV infection (Turner et al., 2014; Frasca et al., 2015; Trzonkowski et al., 2003; Derhovanessian et al., 2013; Reed et al., 2017; Arias et al., 2013; Moro-García et al., 2012; Wald et al., 2013), others no impact (Haq et al., 2017; Derhovanessian et al., 2013; den Elzen et al., 2011; Strindhall et al., 2016), and others reporting a positive impact of CMV on antibody induction (Furman et al., 2015; McElhaney et al., 2015; Berg et al., 2018). While a recent meta-analysis concluded that there was no clear evidence of an association between CMV infection in influenza vaccine induced antibodies (van den Berg et al., 2019), reported differences between studies may be due to the age of individuals (with some suggestion that the negative impact is more important in aged individuals), specific antigen investigated, or methodology. Further, the induction of a primary compared to memory response may also be important. Indeed, a recent study of primary exposure to an Ebola vaccine candidate (chimpanzee adenovirus type-3 vectored Ebola Zaire vaccine, ChAd3-MVA-EBO-Z) showed that latent CMV infection negatively impacted antibody induction (Bowyer et al., 2020). Differences in CMV seroprevalence between recipients of this Ebola vaccine in the UK (50% CMV positive) and Senegal (100% CMV positive) have been proposed to account for the previously reported reduced vaccine responsiveness in the Senegalese cohort, with CMV infected UK participants having comparable responses to Senegalese individuals (Venkatraman et al., 2018).

Reduced responses to *Plasmodium falciparum* malaria vaccine candidates have also been reported in LMIC populations. For example, antibodies to circumsporozoite protein (CSP) following experimental vaccination with radiation-attenuated whole sporozoite parasite vaccine (PfSPZ) were significantly lower in Tanzanian and Malian adults, compared to cohorts in the US (Jongo et al., 2018). Further, CSP antibodies induced in Kenyan adults with licenced malaria subunit vaccine RTS,S are generally lower than those seen following vaccination of US cohorts (Stoute et al., 1997; Kester et al., 2001, 2007; Polhemus et al., 2009). While multiple factors may underpin these geographic variations in malaria vaccine efficacy (van Dorst et al., 2024), to date the potential impact of latent CMV infection on immune responses to malaria is unknown.

To investigate this, we analysed previously published data on the development of antibody responses in malaria-naïve adults enrolled in blood stage controlled human malaria infection (CHMI) studies (Chan et al., 2020). We show that CMV infection was associated with reduced antibody induction following CHMI. CMV infected individuals had bias within the T-follicular helper (Tfh) CD4 T cell compartment towards Tfh1 cell types, which were negatively associated with antibody induction. Together, these data show CMV infection is a significant modulator of the adaptive host immune response to *P. falciparum* malaria.

Results and Discussion

Latent CMV infection is associated with reduced antibody induction following controlled human malaria infection in adults.

To investigate the impact of CMV infection on the immune response to *P. falciparum* infection, we reanalysed published data of 40 malaria-naïve adults during blood stage CHMI (median age 25.5, range 18-52 years) (Chan et al., 2020). Within the cohort, 21/40 (52%) individuals were sero-positive for CMV. Sex, Epstein-Barr virus (EBV) infection, and age were comparable between the CMV negative and positive individuals (Table 1).

Table 1: Study populations demographics

	CMV serostatus		
	Negative	Positive	P
Total, n (%)	19, (48%)	21, (52%)	0.751*
Sex, male, n (%)	18 (95%)	18 (86%)	1*
EBV, positive, n (%)	18 (95%)	17 (81%)	0.865*
Age years, median [IQR]	26 [20.25-31.75]	25 [21-29]	0.86#

* Chi-square, # wilcox rank sum

As previously reported, the induced antibody response was measured as an antibody score that captured the breadth, magnitude and functionality of antibodies to the merozoite stage parasite, and major merozoite antigen merozoite surface protein 2 (MSP2) (Chan et al., 2020). Antibody score was significantly lower in CMV positive individuals (Fig. 1 A). There was no difference in antibody score with sex or EBV status, and antibody score was not associated with age (Fig. 1 B-D).

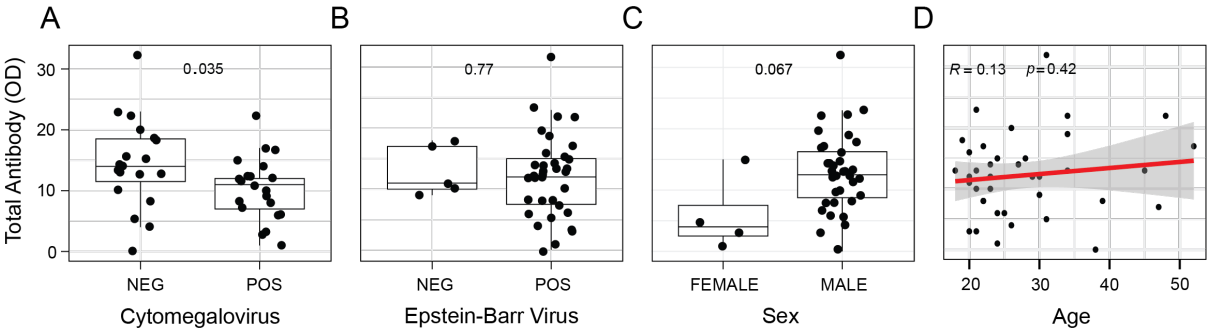

Figure 1. ***Antibodies induced by controlled human malaria infection are reduced in CMV infected adults.***
Total antibodies to merozoite and major merozoite antigen MSP2 were quantified and antibody score used to capture the total magnitude, breadth and functionality of the responses. Antibody score stratified by ***(A)*** *CMV serostatus (CMV- n=19, CMV+ n=21),* ***(B)*** *EBV serostatus (EBV– n=5, EBV+ n=35),* ***(C)*** *Sex (female n=3, male n=37), and* ***(D)*** *correlated with age. A-C data is Tukey boxplots with the median, 25th and 75th percentiles. The upper and lower hinges extend to the largest and smallest values, respectively but no further then 1.5XIQR from the hinge. P are Mann-Whitney U test. D, rho and P are Spearmans correlations.*

CMV seropositive adults have reduced induction of IgG1, and reduced functional antibody responses following controlled human malaria infection

To assess the specific antibodies that were contributing to reduced antibody score in CMV positive individuals, IgG, IgM, specific IgG subclasses and functional antibodies targeting the merozoite were analysed. In CMV positive individuals, IgG to the merozoite was lower at end of study (EOS), but there were no differences in the magnitude of induced IgM (Fig. 2 A). The reduced IgG in CMV positive individuals was driven by a reduced IgG1 response. The magnitude of IgG2 and IgG3 were also lower, however differences were not statistically significant and IgG4 was comparable between groups (Fig. 2 B). IgG1 is a cytophilic antibody which has important functional capacity to fix complement and interact with Fc receptors on phagocytes. These functional antibody responses have essential roles in immunity to malaria and can target the merozoite to block invasion of the red blood cell and mediate protection (Opi et al., 2021; Boyle et al., 2015; Osier et al., 2014). Consistent with reduced IgG1 induction in CMV positive individuals, CMV positive individuals had reduced induction of antibodies that could fix complement (measured by C1q fixation, the first step in the classical complement cascade (Boyle et al., 2015)), and reduced binding of FcγRII and FcgγIII, which are involved in phagocytosis of parasites by neutrophils (Feng et al., 2021) (Fig. 2 C/D). However, antibodies that could mediate opsonic phagocytosis by the THP-1 pro-monocytic cell line, which primarily involves FcγRI (Feng et al., 2021), did not differ between the two groups (Fig. 2 E). There was no difference in the antibody response to MSP2 (Supplementary Fig. 1).

Together these data show that CMV is an important modulator of the primary immune response to malaria infection. This finding is consistent with recent studies evaluating the primary immune response to Ebola vaccination in UK and Senegalese participants (Bowyer et al., 2020). In that study, CMV status was strongly associated with the reduced induction of antibodies following vaccination in Senegalese compared to UK cohorts (Venkatraman et al., 2018; Bowyer et al., 2020). While these differences in UK and Senegalese participants mimic differences in responsiveness to malaria vaccines seen between cohorts in the US and malaria endemic countries (Stoute et al., 1997; Kester et al., 2001, 2007; Polhemus et al., 2009; Jongo et al., 2018), further studies are required to analyse the impact of CMV on malaria vaccine responsiveness. Indeed, multiple factors, alongside CMV status, may influence differing

vaccine efficacy between high and LMIC (van Dorst et al., 2024). While the majority of previous studies evaluating the impact of CMV on immune responses have focused on vaccination, here we show that CMV may also modulate responses to pathogen infection. The negative impact of CMV on antibodies induced during *Plasmodium* infection contrasts with a single study which reported higher antibodies to H1N1 influenza following infection in CMV positive individuals (Nielsen et al., 2015). Further, another study showed no impact of CMV on antibodies induced following SARS-CoV-2 infection (Freeman et al., 2023), suggesting that the impact of CMV on the host immune response to infection is highly pathogen dependent.

Additionally, data suggest that that CMV infection is associated with a reduction in of anti-parasitic antibodies during malaria is not a global response, but instead specifically impacts IgG1 cytophilic subclasses and associated functions. To the best of our knowledge, previous studies on the impact of CMV on heterologous antibody induction have only considered total antibody responses, and have not investigated specific IgG subclasses. The impact seen here on IgG1 subclass responses suggests that the impact of CMV may be specific to subclass switching, rather than early activation of B cells. Indeed, the IgM response to the merozoite did not differ between CMV infected and uninfected individuals in this cohort. Further, the importance of antigen specificity on CMV modulation may contribute to the mixed findings of previous studies.

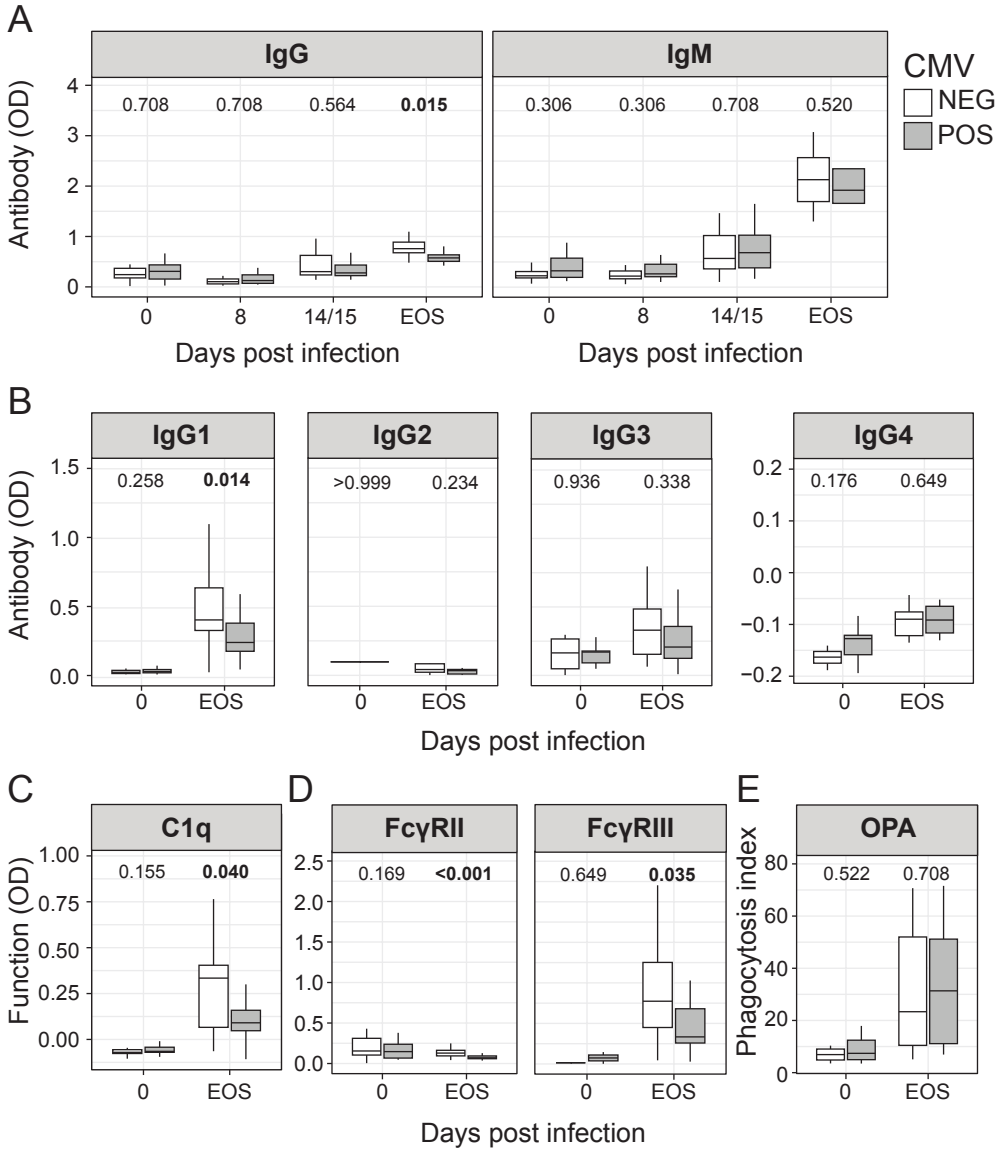

Figure 2. ***IgG1 and functional antibodies to the merozoite induced by controlled human malaria infection are reduced in CMV infected adults***
(A) *IgG and IgM antibodies,* ***(B)*** *IgG subclass antibodies and levels of functional antibodies that can* ***(C)*** *fix C1q complement component,* ***(D)*** *cross link FcgRII and FcgRIII, and* ***(E)*** *drive opsonic phagocytosis by THP1 cells, targeting merozoite stage parasites, stratified by CMV serostatus. CMV- white bars (n=19), CMV+ grey bars (n=21), data is Tukey boxplots with the median, 25th and 75th percentiles. The upper and lower hinges extend to the largest and smallest values, respectively but no further then 1.5XIQR from the hinge. P are Mann-Whitney U test. See also Supplementary Figure 1.*

CMV seropositive adults have expansion of Tfh1 cells, and reduced proportions of activated Tfh cells with Tfh2 phenotypes during infection

Antibody production during infection and vaccination is supported by specialised CD4 T cells, T-follicular helper (Tfh) cells, which activate naïve and memory B cells to drive germinal center activation and antibody development (Crotty, 2019). Tfh cells have essential roles in antibody development during malaria (Soon et al., 2021). In human infection, Tfh cell subsets can be identified based on CXCR3 and CCR6 chemokine expression (Tfh1 CXCR3+CCR6-, Tfh2 CXCR3-CCR6-, Tfh17 CXCR3-CCR6+). Specific Tfh cell subsets are associated with the development of antibodies following infection and vaccination in a context dependent manner, with different subsets associated with antibody induction for different infections or vaccinations. These differing functional potentials may be underpinned by cytokine skewing of Tfh cells (Olatunde et al., 2021). We have previously shown that in this cohort of CHMI participants, early activation of Tfh2 cells was associated with antibody induction (Chan et al., 2020). In contrast, activation of Tfh1 cells was associated with increased antibody secreting cell development which may impair germinal centres by acting as a nutrient sink during infection (Vijay et al., 2020). To assess if CMV infection impacted Tfh cell differentiation during CHMI, we analysed the same data set, and stratified by CMV serostatus. Tfh cells were defined as all CXCR5+ cells, subsets identified based on CXCR3 and CCR6 expression, and activation measured by expression of PD1, CD38 and ICOS. Non-Tfh, effector CD4 cells (CXCR5- CD4 T cells) were also analysed (Supplementary Fig. 2 A). Within the CD4 T cell population, there was no difference in the proportion of Tfh cells (CXCR5+ % of CD4 T cells), nor any major differences in CD4 effector (CXCR5-) Th1-, Th2- and Th17- like populations between CMV infected and uninfected individuals (Supplementary Fig. 2 B-C). Further, there was no difference in magnitude of activation in Tfh cells, nor non-Tfh effector cells during CHMI aside from an increase in Th2 cell activation at day 14 post-inoculation (Fig. 3 A, Supplementary Fig. 2 D-E). However, within the Tfh cell population, CMV infected individuals had a significantly higher proportion of Tfh1 cells, and a significantly decreased proportion of Tfh2 cells, before and during CHMI (Fig. 3 B). This expansion towards Th1-like cells was not seen in non-Tfh effectors, suggesting that the expansion of Tfh1 in CMV infected individuals was not due to a systemic inflammatory phenotype (Supplementary Fig. 2 C). As a result of Tfh1 expansion, amongst activated Tfh cells (either PD1+, ICOS+ or CD38+ Tfh cells), there was a significantly increased proportion of Tfh1 cells and a reduced proportion of Tfh2 cells at day 14/15 (Fig. 3 C). Amongst CMV infected individuals, the proportion of Tfh1 cells in the Tfh cell compartment negatively correlated with antibody score (Fig. 3 D). In contrast, the activation of Tfh2 cells, previously shown to be associated with antibody induction (Chan et al., 2020),

was only associated with antibody score in CMV negative individuals (Fig. 3 E). Together, these data suggest that latent CMV infection modulates the Tfh cell compartment, which reduces antibody production during malaria.

Within this CHMI cohort, we have previously shown that the activation of Tfh2 cells at peak parasitemia was associated with antibody induction (Chan et al., 2020). However, here we show that this association was only detected in CMV negative individuals. In contrast, in CMV infected individuals, the Tfh compartment was skewed to Tfh1 cells, and the magnitude of this skew was negatively associated with antibody induction. The roles of specific Tfh subsets are thought to have depend on the disease or vaccination setting (Olatunde et al., 2021). As such, the CMV-driven skewing of Tfh to Tfh1 subsets will likely have differing effects depending on the infecting pathogen or vaccination, and the relative importance for Tfh1 cells in antibody induction compared to other Tfh cell phenotypes. Indeed, in response to influenza vaccination, studies have shown a positive association between Tfh1 cells and antibody induction (Bentebibel et al., 2013, 2016), which may contribute to the positive association of CMV infection with influenza vaccination responses reported in some studies (Furman et al., 2015; McElhaney et al., 2015; Berg et al., 2018). Further, studies have suggested that Tfh1 cells are robust activators of memory B cells, but have relatively reduced capacity to activate naïve B cells compared to Tfh2 subsets (Bentebibel et al., 2013). CMV driven expansion of Tfh1 cells may have a negative impact on driving a primary immune response, but a positive impact on activating memory B cells. Regardless, Tfh2 cells have been associated with production of antibodies induced by malaria vaccination with both the licenced RTS,S (Bowyer et al., 2018) and experimental blood stage malaria vaccines (Minassian et al., 2021; Nielsen et al., 2021). As such, CMV driven skewing of Tfh cells to Tfh1 phenotypes may also reduce malaria vaccine responses.

Interestingly, within our cohort, the bias towards Tfh1 cell development was not mirrored by increased proportions of Th1-effector cells, suggesting a Tfh cell specific effect. While the antigen specificity of cells was not assessed, in previous studies it has been shown that CMV antigen specific Tfh cells are dominated by Tfh1 cell subsets (Niessl et al., 2020). Further, following SARS-CoV-2 mRNA vaccination, higher frequencies of spike antigen specific Th1 cells were detected in CMV positive individuals (Breznik et al., 2022). This study did not include CXCR5 to assess Tfh cell populations, and therefore the increased Th1 cell population could include Tfh1 cell subsets, consistent with our data. We have previously

shown that in naturally acquired malaria infection in Indonesians, both Tfh1 and Tfh2 malaria specific cells are detected during infection (Oyong et al., 2022). However, the effect of CMV infection on Tfh malaria specific cells is unknown and will require further study.

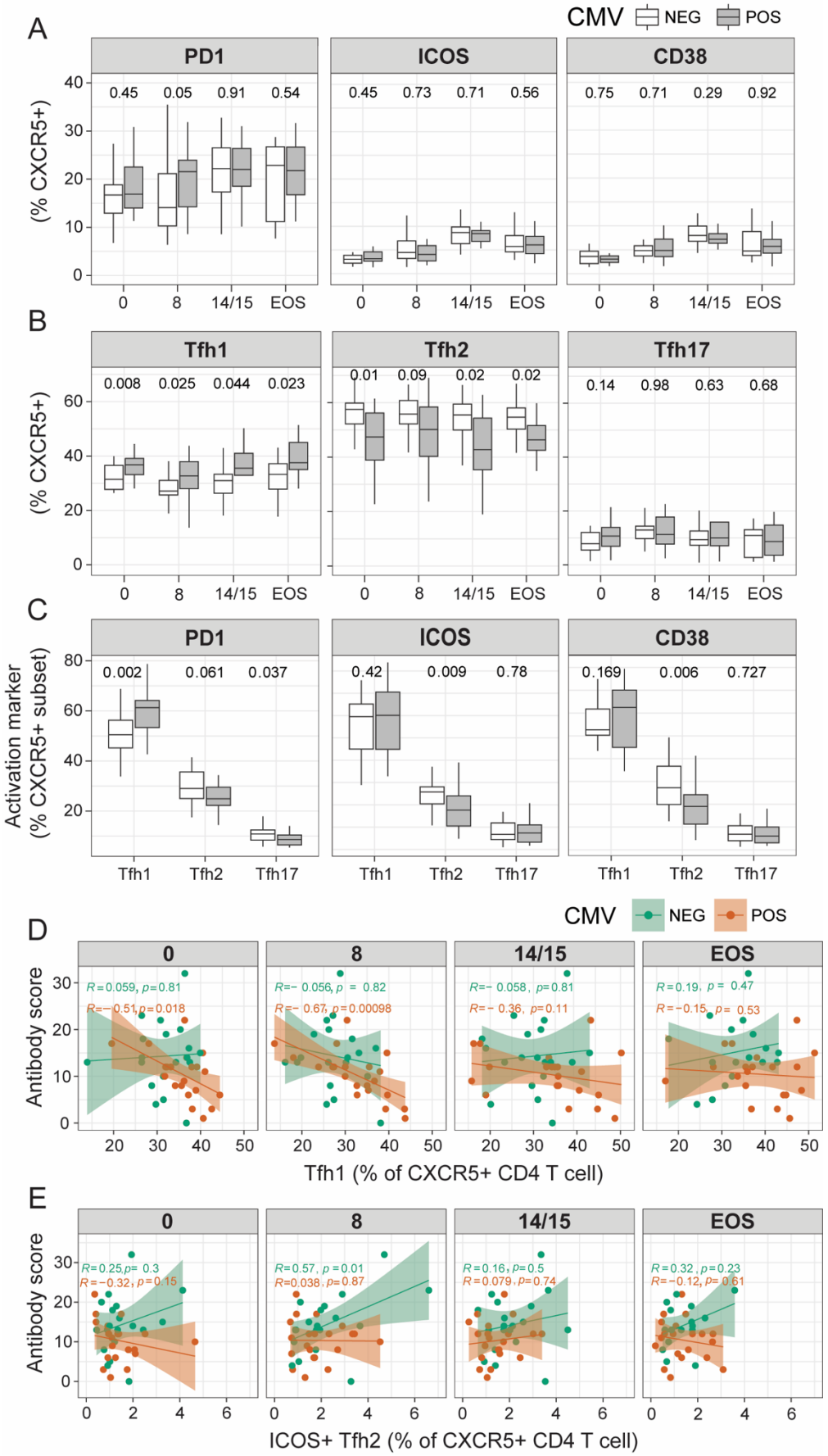
A
CMV NEG POS
PD1
ICOS
CD38
(% CXCR5+)
0.45 0.05 0.91 0.54
0.45 0.73 0.71 0.56
0.75 0.71 0.29 0.92
0 8 14/15 EOS
B
Tfh1
Tfh2
Tfh17
0.008 0.025 0.044 0.023
0.01 0.09 0.02 0.02
0.14 0.98 0.63 0.68
C
Activation marker (% CXCR5+ subset)
0.002 0.061 0.037
0.42 0.009 0.78
0.169 0.006 0.727
Tfh1 Tfh2 Tfh17
D
CMV NEG POS
0
8
14/15
EOS
Antibody score
R=0.059, p=0.81
R=−0.51, p=0.018
R=−0.056, p=0.82
R=−0.67, p=0.00098
R=−0.058, p=0.81
R=−0.36, p=0.11
R=0.19, p=0.47
R=−0.15, p=0.53
Tfh1 (% of CXCR5+ CD4 T cell)
E
R=0.25, p=0.3
R=−0.32, p=0.15
R=0.57, p=0.01
R=0.038, p=0.87
R=0.16, p=0.5
R=0.079, p=0.74
R=0.32, p=0.23
R=−0.12, p=0.61
ICOS+ Tfh2 (% of CXCR5+ CD4 T cell)

Figure 3. ***Tfh cell response is skewed to Tfh1 subsets in CMV infected adults***
(A) *Activation of Tfh cells as measured by PD1+, ICOS+, and CD38+ cells (% of Tfh CD4 T cells) in CHMI stratified by CMV status.* ***(B)*** *Tfh cell subsets (% of Tfh CXCR5+ CD4 T cells), stratified by CMV status.* ***(C)*** *Tfh cell subsets as a proportion of activated Tfh cells, stratified by CMV serostatus at day 14/15.* ***(D)*** *Correlation between the proportion of Tfh1 (% of Tfh CXCR5+ CD4 T cells) or* ***(E)*** *the proportion of activated Tfh2 cells (ICOS+ Tfh2 (% of Tfh CXCR5+ CD4 T cells) at day 0, 8, 14/15, EOS and Antibody score measured at EOS. For* ***A-C*** *CMV- white bars (n=19 for day 0, 8, 15, n=17 for day EOS), CMV+ grey bars (n=21 for day 0, 8, 15 and n=20 for EOS), data is Tukey boxplots with the median, 25th and 75th percentiles. The upper and lower hinges extend to the largest and smallest values, respectively but no further then 1.5XIQR from the hinge. P are Mann-Whitney U test. See also Supplementary Figure 2.*

Concluding remarks

Taken together, these data show that latent CMV infection has a striking negative effect on the development of malaria-specific antibody production after CHMI. This reduced antibody induction was linked to the skewing of Tfh cells to the Tfh1 cell subsets, and away from Tfh2 cells, which have previously been linked to antibody induction in CHMI (Chan et al., 2020). These findings have important implications for understanding immune responses to malaria infection and vaccination. In malaria endemic areas, a large proportion of infants are infected by CMV within the first year of life (Kaye et al., 2008; Miles et al., 2008). As this time period overlaps with first exposures to malaria parasite infection, further studies are required to assess if CMV infection contributes to the slow acquisition of protective functional antibodies in children in malaria endemic areas. Tfh2 cells have also been associated with antibody induction in response to the RTS,S malaria vaccine (Bowyer et al., 2018). These antibody responses are influenced by geographic location, being lower in Kenyan compared to US cohorts (Stoute et al., 1997; Kester et al., 2001, 2007; Polhemus et al., 2009). Whether differences in latent CMV infection underpin these findings warrant further investigation.

Materials and Methods

Study populations

Written informed consent was obtained from all participants. Ethics approval for the use of human samples in the relevant studies was obtained from the Alfred Human Research and Ethics Committee for the Burnet Institute (#225/19), and from the Human Research and

Ethics Committee of the QIMR-Berghofer Medical Research Institute (P1479, P3444 and P3445).

Controlled human malaria infection studies were performed as previously described using the induced blood stage malaria model (McCarthy et al., 2011b). Malaria naïve individuals were inoculated by intravenous injection of 2800 *P. falciparum* infected red blood cells and monitored for parasite growth with qPCR (Rockett et al., 2011). Here, blood samples were collected at baseline (day 0), day of treatment (day 8) and at 14 or 15 days and at end of study (EOS), 27-36 days after inoculation (4 studies, across 6 independent infection cohorts). Clinical trials were registered at ClinicalTrials.gov NCT02867059 (Gaur et al., 2020), NCT02783833(McCarthy et al., 2020) , NCT02431637 (Collins et al., 2018), NCT02431650 (Collins et al., 2018). Whole blood was stained and analysed immediately by flow cytometry and plasma was collected from lithium heparin collection tubes.

CMV and EBV serostatus

CMV and EBV seroprevalence was assessed using baseline plasma samples by commercially available ELISA kits (ab108724 and ab108730), according to manufacturer's instructions. CMV ELISA kit has a reported sensitivity and specificity of 98% and 97.5% respectively.

Induced antibody responses

Antibody response to intact merozoites and merozoite surface antigen MSP2 were quantified as previously described (Chan et al., 2020). To isolate merozoites, *P. falciparum* 3D7 parasites were maintained in continuous culture in RPMI-HEPES medium supplemented with hypoxanthine
(370 mM), gentamicin (30 mg/ml), 25 mM sodium bicarbonate and 0.25% AlbuMAX II (GIBCO) or 5% heat-inactivated human sera in O+ RBCs from malaria-naive donors (Australian Red Cross blood bank). Cultures were incubated at 37°C in 1% O_2, 5% CO_2, 94% N_2 and schizont stage parasites were purified by MACS separation (Miltenyl Biotec). To isolate merozoites, magnet purified schizonts were incubation with the protease inhibitor E64 (10 mg/ml), and following complete development, merozoites were isolated by membrane filtration (1.2 mm). 50 ml of *P. falciparum* 3D7 merozoites (2.5 X 10^5 merozoites/ml) or 50 ml of 0.5 mg/ml MSP2 recombinant antigen (McCarthy et al., 2011a) in PBS were coated to 96-well flat bottom MaxiSorb plates (Nunc) overnight at 4°C. Plates were blocked with 10% skim milk for merozoites, or 1% casein (Sigma-Aldrich) for MSP2 for 2 hours at 37°C. Plasma was diluted in 0.1% casein in PBS

1/100 for IgG, 1/250 for IgG subclasses and IgM, 1/100 for C1q, 1/100 for FcγR targeting merozoites or 1/50 for FcγR targeting MSP2) and incubated for 2 hours at room temperature. For total antigen specific IgG detection, plates were incubated with goat polyclonal anti-human IgG HRP- conjugate (1/1000; Thermo Fisher Scientific) for 1 hour at room temperature. For detection of IgG subclasses and IgM, plates were incubated with a mouse anti-human IgG1 (clone HP6069), mouse anti-human IgG3 (HP6050) or mouse anti-human IgM (clone HP6083) at 1/1000 (Thermo Fisher Scientific) for 1 hour at room temperature. This was followed by detection with a goat polyclonal anti-mouse IgG HRP-conjugate (1/1000; Millipore). For all ELISAs, plates were washed three times with PBS (for merozoite ELISAs) or PBS-Tween 0.05% (for MSP2) between antibody incubation steps. For detection of complement fixing antibodies, following incubation with human sera, plates were incubated with purified C1q (10 mg/ml; Millipore) as a complement source, for 30 min at room temperature. C1q fixation was detected with rabbit anti- C1q antibodies (1/2000; in-house) and a goat anti-rabbit-HRP (1/2500; Millipore). For FcγR assays, 100ul of biotin-conjugated rsFcγRIIa H131 or rsFcγRIIIa V158 ectodomain dimer (0.2ug/ml) was incubated at 37°C for 1 hour followed by 3 washes with PBS-Tween. The binding was detected with horseradish peroxidase (HRP)-conjugated streptavidin antibody (1:10,000) in PBS-BSA at 37°C for 1 hour.
TMB liquid substrate (Life Technologies) was added for 1 hour at room temperature and the reaction was stopped using 1M sulfuric acid. The optical density (OD) was read at 450 nm. For opsonic phagocytosis, THP1 cells were incubated with intact merozites (stained with Ethidium Bromide and opsonised with plasma diluted 1/100) or latex beads coated with MSP2 (opsonised with plasma diluated 1/10) for 20 min at 37°C and cells washed with FACS buffer. The proportion of THP-1 cells containing fluorescent-positive beads was evaluated by flow cytometry (FACS CantoII, BD Biosciences), analyzed using FlowJo software and presented as phagocytosis index (the percentage of THP-1 monocytes with ingested merozoites or beads).

To calculate antibody score antibody responses below positive cut-off threshold were set as negative, and remaining positive responses were used to calculate median and used to categorise responses into low (below median) and high (above median) responses. Antibody score was calculated by giving categories zero/low/high a numerical score of 0/1/2 and then summing across all antibody responses.

Quantification of T cells

Whole blood was analysed as described previously (Chan et al., 2020). 200 mL of whole blood were stained with the following conjugated antibodies, all from BD Biosciences; anti-CD20-BUV395 (2H7, 1:150, Cat# 563782), anti-CXCR5-BV421 (RF8B2, 1:50, Cat#562747), anti-CD4 (V500, 1:30, Cat# 560768), anti-CCR6-BV650 (11A9, 1:200, Cat#563922), anti-CD38-BV786 (HIT2, 1:400, Cat#563964), anti-CXCR3-APC (1C6, 1:25, Cat#550967), anti-CD27-APC-R700 (M-T271, 1:100, Cat#565116), anti-CD8-APC-Cy7 (SK1, 1:150, Cat#557834), anti-CD19-FITC (HIB19, 1:20, Cat#55412), anti-CD45-PerCP-Cy5.5 (2D1, 1:50, Cat#340953), anti-ICOS-PE (DX29, 1:10, Cat#557802), anti-CD3-PE-CF594 (UCHT1, 1:600, Cat#562280), anti-PD1-PE-Cy7 (EH12.1, 1:100, Cat#561272). RBCs were lysed with FACS lysing solution (BD) and re-suspended in 2% FBS/PBS. Samples were acquired on the BD LSR Fortessa TM 5 laser cytometer (BD Biosciences). These data were analyzed using FlowJo version 10.8 software (Tree Star, San Carlos, CA, USA).

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Authors declare no conflicts of interest.

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