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REVIEW

Maternal immune correlates of protection from human cytomegalovirus transmission to the fetus after primary infection in pregnancy

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Summary

Immune control of primary human cytomegalovirus (HCMV) infection appears to depend on the interaction of humoral and T-cell responses. In this review, we have separately explored the 2 arms of the immune response to primary HCMV infection in HCMV-seronegative pregnant women transmitting (T) or not transmitting (NT) the infection to the fetus, with the objective of correlating the immune risk factors associated with vertical HCMV transmission. As for the humoral response, the following findings were documented: (i) in competitive binding assays, antibody titers to different antigenic sites of the gH pentamer complex were significantly lower in T compared with NT women; (ii) in addition, the number of neutralization sites recognized by T was significantly lower compared with NT women; (iii) the plaque formation inhibition assay showed a faster kinetics and a higher titer in NT women. As for T-cell immunity, the delayed expression of 3 immunological parameters (lymphoproliferative response, CD45RA reexpression in both $CD4^+$ and $CD8^+$ HCMV-specific T cells, and interleukin-2 production by HCMV-specific $CD4^+$ T cells) were significantly associated with vertical transmission. This overview provides important information at the population level, which may help to inform the evaluation of interventions such as vaccination or treatments designed to interrupt intrauterine transmission of HCMV during primary infection. However, although we are waiting for an HCMV vaccination to become available, we emphasize that none of these parameters can be prognostically used on an individual basis because of the great variation in values among women.

KEYWORDS

congenital infection, human cytomegalovirus, immune protection, pregnancy, primary infection

1 | INTRODUCTION

Immune control of human cytomegalovirus (HCMV) and, in particular, primary HCMV infection appear to depend on humoral and (innate and adaptive) cell-mediated immune response interactions. Because the control of HCMV infection is multifactorial, the sole presence of an efficient virus-specific antibody response, as observed frequently in immune-suppressed transplant recipients in the absence of a specific T-cell response,[1–4] does not prevent severe systemic HCMV infections, and the administration of hyperimmune globulin preparations to seronegative pregnant women undergoing primary HCMV infection[5,6] does not significantly prevent HCMV transmission to the fetus.[7] Recently, we had the opportunity to verify that in immune-suppressed transplant recipients, the antibody response was significantly higher than that found in immune-competent subjects, starting 6 months after onset of infection.[4] This elevated antibody response was observed in the absence of protection from HCMV infection, with titers never previously observed in immune-competent individuals, ie,

Abbreviations: AI, avidity index; gB, glycoprotein B; gCs, glycoprotein complexes; HCMV, human cytomegalovirus; HELF, human embryonic lung fibroblasts; IE, immediate-early; IL-2, interleukin-2; IL-7R, interleukin-7 receptor; IMAB, inhibition of monoclonal antibody binding; LPR, lymphoproliferative response; LTI, leukocyte transfer inhibition; NT, nontransmitting mothers; Pentamer, gH/gL/pUL 128, 130, 131 or gH/gL/pUL128L; PFI, plaque formation inhibition; SFI, syncytium formation inhibition; T, transmitting mothers; T_{EM}, effector memory T cells; T_{EMRA}, effector memory RA^+ T cells; Trimer, gH/gL/gO; α/ß T cells, alfa/beta T cells; γ/δ T cells, gamma/delta T cells

titers ranging from 1×10^5 to 1×10^6 for both HCMV-specific ELISA IgG to viral glycoprotein complexes (gCs) and neutralizing antibodies preventing infection of epithelial cells.[8–10] This exaggerated antibody response was observed before reconstitution of HCMV-specific $CD4^+$ T cells. As a result, the interaction of humoral and cell-mediated immunity in the protection process requires further study.

In the last decade, our group has been investigating several aspects of the immune response (both humoral and T-cell mediated) to primary HCMV infection in pregnant women, with special reference to the risk of virus transmission to the fetus. Results of these studies will be the subject of this review, where maternal immune correlates of virus transmission to the fetus will be discussed in light of our group's experience and the existing literature.

2 | HUMORAL IMMUNITY

2.1 | Broad antibody response to whole HCMV virus particles

In primary HCMV infection, total HCMV-specific IgG antibodies to HCMV lysate increase, whereas immunoglobulin M (IgM) antibodies to HCMV lysate as well as HCMV DNA load during the first 3–6 months after infection decrease. In parallel, antibodies neutralizing the infection of ARPE-19 epithelial cells appear early and rapidly reach high titers, whereas serum antibodies able to neutralize infection of human embryonic lung fibroblasts (HELF) appear later and peak at approximately 6 months after onset of infection, with titers that are 2–3 $\log_{10}$ lower.[11] Recently, it has been shown that the presence of overt clinical symptoms is associated with a significantly higher antibody response (concomitantly with a higher viral load) in nonpregnant subjects with symptomatic primary HCMV infection as compared with pregnant women with paucisymptomatic/asymptomatic primary infection (and lower viral load).[12] In addition, in the former patient group, the IgG response to HCMV lysate and the ARPE-19 neutralizing antibody response are significantly higher from day 30 through day 180 after infection onset, whereas the HELF-neutralizing antibody response is significantly higher starting 90 days postinfection.

However, no significant difference either in the kinetics or peak level of whole virus lysate IgG and IgM antibodies was observed between the group of women transmitting (T) and that of women not transmitting (NT) the virus to the fetus.[11] Different results were obtained when IgG absorbance in the presence or absence of urea and the IgG avidity index (AI) were determined.[13] The percent increase in avidity values (absorbance in the presence of urea) between early (T1, 0-90, median 31 days) and late (91-180, median 136 days) serum samples was calculated for each of the 69 pregnant women with primary infection. In this way, 3 groups of women were identified: those with a high (100%) avidity increase between T1 and T2 (pattern H), those with a low (<50%) avidity increase (pattern L), and finally those with an intermediate avidity increase (pattern I). In detail, in pattern H, avidity in the presence of urea was found to increase over time, whereas in pattern L, it remained substantially unchanged. In addition, in the absence of urea, the absorbance values in pattern H were high and did not vary during the entire follow-up period, whereas they slowly decreased over time with pattern L. As a result, a steady maturation process from low to high AI was observed in women with pattern H, whereas in women with pattern L, the maturation was less evident, showing a significantly lower slope. However, the most remarkable finding was that vertical transmission occurred at a significantly higher rate in women with pattern H (62.5%) compared with women with pattern L (24.1%). The conclusion was that women with a slower maturation of IgG avidity appeared less prone to transmit the infection to the fetus (Figure 1).

This result was recently confirmed by another study showing that a rapid increase in the serum HCMV IgG AI in pregnant women with primary infection was associated with delivery of congenitally infected newborns.[14]

Several commercial assays are now available for measurement of HCMV IgG AI, and their performance has been repeatedly evaluated in different laboratories.[15–17] In addition, the kinetics of an IgM antibody level decrease may also help to define the course of IgG avidity maturation, in that the highest IgM antibody levels have been reported to be associated with the lowest AI values.[18]

2.2 | Specific antibody response to HCMV gCs

Three major gCs are present on the envelope of HCMV virus particles: gCI, gCII, and gCIII.[19] gCI consists of a glycoprotein B (gB) homotrimer, gCII of gM/gN, and gCIII of gH/gL complexed with additional proteins. Briefly, UL55-coded HCMV gB forms a trimer (gCI) on the viral envelope, which mediates fusion during viral entry and was considered in the past the main target of neutralizing antibody, undergoing conformational changes when the virion envelope fuses with the target cell membrane. It is important for both cell-to-cell spread and cell-cell fusion (leading to syncytium formation). Although several candidates have been investigated, no cellular receptor for gB has yet been identified. Natural infection elicits neutralizing antibodies directed to gB. On this basis, a gB-based vaccine has been formulated and clinically tested to prevent HCMV infection and disease. However, results were only partially satisfactory.[20,21] Incomplete protection has also been reported after administration of hyperimmune globulin preparations with high gB-specific antibody binding titers in both solid-organ transplant recipients[22] and congenitally infected infants.[23]

The second envelope glycoprotein complex (gCII) consists of UL100-coded gM and UL73-coded gN. gM is the most abundant envelope glycoprotein, but only a small portion of gM is required for gM/gN complex formation. gN is one of the most variable gene products, and has been used to track viral strains in clinics.[24]

The third envelope glycoprotein complex (gCIII) consists of UL75-coded gH and UL115-coded gL. The gH/gL complex associates with additional proteins, which act as tropism determinants. UL74-encoded gO may be complexed with gH/gL, thus forming the trimeric complex gH/gL/gO, which binds to PDGFRα and mediates HCMV entry into HELF.[25] In addition, with the proposed activation of gB fusion machinery, it could also be involved in entry into all cell types. On the other hand, gH/gL may be complexed with the UL128/130/131 locus (UL128L) gene products, thus giving rise to the pentameric complex gH/gL/pUL128L. Although gH and gL have been previously characterized, the UL128L locus has more recently been identified as being indispensable for HCMV entry and growth in endothelial cells and for

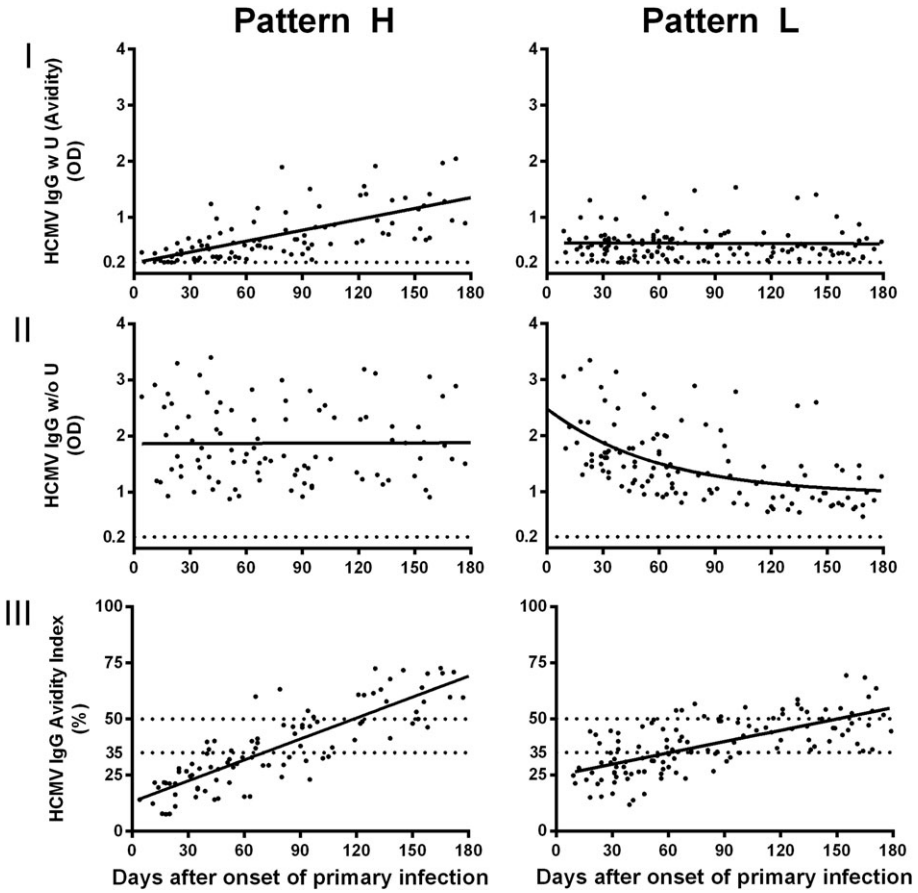

FIGURE 1 HCMV ELISA absorbance (OD) of IgG antibody after urea treatment (IgG + U, panels I H, and L), IgG antibody without urea treatment (IgG-U, panels II H, and L), and IgG avidity index (AI, panels III H, and L) during the follow-up period in 24 women with pattern H and 29 women with pattern L. Discontinuous horizontal lines indicate the ELISA IgG cutoff in panels I H and L and II H and L, and they indicate the upper limit of the low (35%) and intermediate (50%) AI in panels III H and L, respectively. Patterns H and L were different in the presence of urea (I) and in the absence of urea (II), whereas the linear regression curves representing the kinetics of IgG AI in patterns III H and L had a significantly different slope ($P < .001$, extra sum-of-squares of F test). (From M. Furione et al., *J Med Virol* 2013; 85: 1960-1967)

virus transfer to leukocytes.[26] Subsequently, Wang and Shenk[27] showed that the UL131 open reading frame (ORF) is required for epithelial cell tropism and proposed that the tetrameric complex gH/gL/pUL128/pUL130 is required for infection of both epithelial and endothelial cells, but not fibroblasts.[28] Finally, Ryckman et al[29] identified the pentamer gH/gL/pUL128L as the entry complex for epithelial/endothelial cells, suggesting the presence of a type-specific receptor. Mass spectrometry and mutagenesis analysis revealed that the introduction of a double mutation at the disulfide bond of the pentamer with gL (UL128-Cys162Ser/gL-Cys144Ser) reduced interference with HCMV entry into epithelial cells.[30] As a result, gH/gL/gO and the pentamer were considered to represent 2 mutually exclusive cell entry complexes. However, gH/gL/gO was also found to be required for viral entry into epithelial cells.[25,31,32]

The first observations on the antibody response to gCI (gB) were published more than 20 years ago, based on the early finding that women with HCMV mononucleosis and those with subclinical infection transmitting the virus to the fetus had a higher and more prolonged serum antibody response than those with subclinical infection not transmitting HCMV in utero.[33] It was reported that anti-gB IgG antibody titers were significantly higher at delivery in T than in NT mothers, thus suggesting that the antibody titer did not correlate with protection from transmission, but neutralizing antibody titer did. In addition, a significant correlation between neutralizing titers and anti-gB IgG avidity was found, suggesting that a defect in IgG avidity maturation may play a role in HCMV intrauterine transmission.[34] These results were subsequently confirmed by showing that a lack of HCMV gB-specific antibodies correlated with a lower risk of intrauterine fetal infection.[35]

More recently, the identification of the 2 gH/gL gCs (gH/gL/gO and gH/gL/pUL128L) has prompted the study of the ELISA antibody response to the 2 gCIII as well as to gCI (gB); in parallel, the serum activity neutralizing the infection of epithelial/endothelial cells and HELF was investigated.[8,10] The study of the ELISA-specific IgG antibody kinetics to soluble forms of the pentamer complex, gH/gL dimer, and gB (produced in transfected cells) in a group of pregnant women during the first year after onset of primary infection showed that antibodies to gB increased rapidly and to a significantly higher titer compared with those against the pentamer, whereas antibodies to gH/gL showed a significantly slower kinetics.[11] In addition, serum antibodies neutralizing the infection of epithelial cells appeared early and rapidly reached higher titers, whereas those neutralizing the infection of HELF appeared much later, reaching titers 2 to 3 $\log_{10}$ lower. On the basis of a fair degree of correlation between anti-pentamer ELISA IgG

antibodies and epithelial cell neutralizing antibody titer, convalescent-phase sera from primary HCMV infections were absorbed with purified gCs. As a result, sera preabsorbed with the pentameric complex showed a highly reduced capacity to neutralize infection of epithelial cells (>90%), whereas their neutralizing capacity was substantially unaffected in convalescent-phase sera preabsorbed with gH/gL or gB.

Subsequently, when a group of NT mothers was compared with a group of T mothers, it was found that although gB antibody titers were not significantly different in the 2 groups, IgG antibody titers against the pentamer and the dimer were significantly higher in NT compared with T pregnant women in the first 30 days after onset of infection. In parallel, viral load was significantly higher in T compared with NT women.[11] Vertical transmission to the fetus was more deeply investigated by taking advantage of the competitive ELISA inhibition of monoclonal antibody binding (IMAB) assay, which had been previously developed for human immunodeficiency virus-1 (HIV-1)[36] and where the pentamer bound to the solid phase is reacted competitively with human sera and murinized human monoclonal antibodies (mAbs).[10] Using the IMAB assay, it was first shown that (1) all blood donors with remote HCMV infection were reactive with all 10 neutralization (antigenic) sites identified by using a panel of neutralizing mAbs targeting distinct sites on the pentamer complex[37] and (2) individuals differ in their production of antibodies to the different sites of the pentamer complex. Then, the IMAB titers in 2 groups of T and NT women examined according to 3 time intervals (<30, 31-60, >60 days after onset of infection) were shown to be significantly lower in the T group as compared with the NT women for 7/10 sites investigated.[11] In addition, the number of neutralization sites recognized by T women was significantly lower than the number of sites recognized by NT women during both the first and the second month after onset of infection, whereas no difference was found in the >60-day interval.

Finally, the blocking activity of human mAbs and convalescent-phase human sera from primary HCMV infections was investigated in T and NT pregnant women with respect to viral dissemination occurring through the following biological events: (i) plaque formation (cell-to-cell spreading) inhibition (PFI), (ii) leukocyte transfer inhibition (LTI), and (iii) syncytium formation inhibition (SFI). HCMV PFI and LTI were first reported some years ago,[8] whereas SFI has been reported just recently.[38] As a general trend, it was found that LTI antibodies[10] and SFI antibodies[38] appeared late (at least 30 days after onset) during the convalescent phase of primary infection, whereas PFI antibodies were detected much earlier. This is likely the reason the PFI_{50} antibody titer was significantly higher in NT women during the first month after onset and almost reached significance in the second month after onset.[11] Similarly, the average kinetics of the PFI_{50} titer (regression curves) increased significantly in NT women and reached a significantly higher titer as compared with T women. Among mAbs, this inhibitory effect on epithelial cells was restricted to neutralizing mAbs directed against the pentameric complex, whereas it was much more reduced for anti-gB and anti-gH mAbs.

Although the differential inhibitory activity in the 2 groups of T and NT women remains for the moment limited to PFI antibodies because of their earlier appearance, it is quite possible that the 3 types (PFI, LTI, and SFI) of inhibiting antibodies (neutralizing mAbs and neutralizing antibodies of convalescent sera from primary infections) contribute significantly to limit virus dissemination through 3 different pathogenetic mechanisms: cell-to-cell spreading, virus transfer to leukocytes from infected endothelial cells of the vascular tree, and syncytium formation. As for the syncytial formation mechanism, it has recently been shown for HIV that more than 50% of virus dissemination occurs cell-to-cell rather than through cell-free virus infection.[39]

3 | INNATE IMMUNITY

Natural killer (NK) cells are the most actively involved innate immune cells during HCMV infection. As a result, a stable expansion of NK cells $NKG2C^{bright}$ occurs.[40] This NK cell subset is found after primary infection early in life, as well as in congenitally infected newborns and in both solid-organ and hematopoietic stem cell transplant recipients.[41–44] NK cells are considered the main effectors of antibody-dependent cell-mediated immune responses. In a recent unpublished study conducted in our laboratory on a group of 15 adult subjects with primary HCMV infection followed for almost 2 years postinfection (pi) and a group of 16 HCMV-seropositive subjects with remote (>5 years) infection as well as a group of 10 HCMV-seronegative subjects, it was observed that, for $CD56^{dim}CD16^{+}$ NK cells, both the absolute number and the percentage of $NKG2C^{bright}CD57^{+}$ cells were significantly higher during primary and remote infection with respect to seronegative subjects (Figure 2). The significance of these findings will be investigated in more detail in the near future to better define their prognostic or diagnostic potential.

In the vaccinology field, antibody titer and neutralizing activity are often the only parameters evaluated for definition of protective immunity. However, often antibody titer and neutralizing antibody activity alone are not sufficient to account for protective immunity.[45] Instead, protective immunity has been observed in the absence of neutralizing activity, while increasing evidence has been accumulating that a critical role in protection from infection belongs to extraneutralizing antibody functions, such as antibody-dependent cellular cytotoxicity,[46] antibody-dependent cellular phagocytosis,[47] antibody-dependent complement deposition,[45] and antibody-dependent NK cell activation.[48]

During natural infection or after vaccination, persisting infections such as malaria, tuberculosis, HIV, and herpesvirus (herpes simplex virus and HCMV) infections develop an extensive array of antibodies targeting different epitopes with different affinities and fragment crystallizable receptor profiles. These contribute to a flexible humoral immune response poised for the elimination of pathogens through mechanisms not involving neutralizing activity. Thus far, the role of antibody-dependent cellular mechanisms involved in the HCMV humoral immune response in pregnant T and NT women has not yet been investigated. However, this is expected to be one of the major fields of investigation in the near future with respect to the role of the maternal immune response to HCMV infection in vertical HCMV transmission.

In addition, γ/δ T cells, which share some properties with both innate and adaptive immune cells, were initially considered a first-line defense mechanism in kidney allograft recipients,[49] based on concomitant γ/δ T-cell expansion and the resolution of HCMV infection/disease.[50] However, only $V\delta2^{-}$ (and not $V\delta2^{+}$) γ/δ T cells undergo

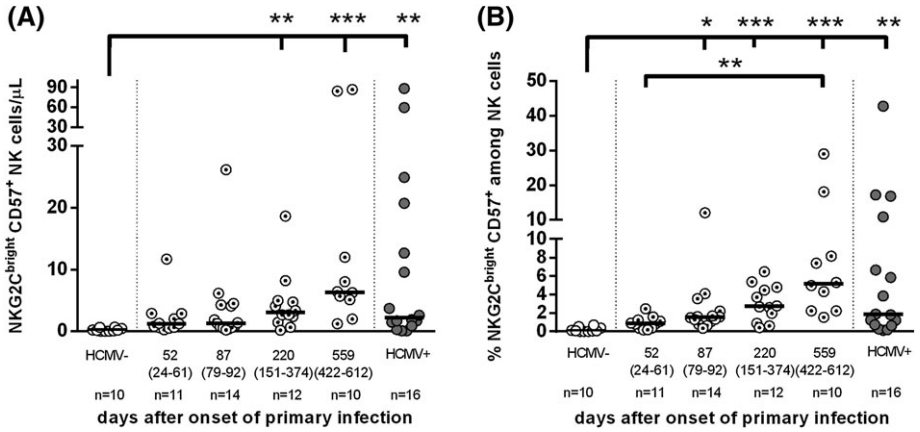

FIGURE 2 A, Number/μL blood of NKG2C^{bright}CD57^{+} NK cells and B, their percentage among NK cells in 10 HCMV-seronegative adult subjects (HCMV^{-}), 15 adult subjects with primary HCMV infection, and 16 HCMV-seropositive subjects with remote (>5 years) infection (HCMV^{+}). Statistically significant differences between subjects with HCMV infection and HCMV-seronegative subjects are shown on the top horizontal line, whereas differences among subjects at different times after primary infection are shown on the lower horizontal line (Kruskal-Wallis test with Dunn's posttest: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$). Blood collection median days (range), after primary infection onset, are reported below the *x* axis

long-lasting expansion and have been found to be able to kill HCMV-infected target cells in vitro.[51] Thus, until recently, γ/δ T cells have been considered as innate effectors unable to establish an antigen-specific memory. However, more recently, the long-term expansion of effector/memory Vδ2^{-} γ/δ T cells has been shown to be a sort of specific signature of the adaptive immune response to HCMV infection in both immune-competent and immune-compromised patients.[52] In addition, in kidney transplant recipients, HCMV-specific CD8^{+} α/β T cells and Vδ2^{-} γ/δ T cells share common expansion kinetics and effector phenotype, suggesting that these cell types act similarly in response to HCMV infection.[53] Furthermore, we have shown that Vδ2^{-} γ/δ T cells in immune-competent pregnant women undergo an expansion kinetics resembling that of transplanted patients.[54] Finally, a strong γ/δ T-cell response to congenital HCMV infection has been shown to develop in utero, suggesting a role in antiviral defense in early life.[55] In conclusion, the higher number of Vδ2^{-} γ/δ T cells observed in HCMV-seropositive subjects as compared with HCMV-seronegative individuals seems to indicate the persistence of a "memory" γ/δ T-cell response or a chronic activation because of intermittent HCMV reactivation. As per the hypothetical role of Vδ2^{-} T cells in HCMV transmission to the fetus, to date no difference in the number/percentage of Vδ2^{-} T cells has been observed between T and NT women.[54]

4 | ADAPTIVE T-CELL IMMUNITY

The evaluation of HCMV-specific T-cell responses to primary infection was performed in our laboratory by measuring the lymphoproliferative response (LPR) to HCMV-infected cell lysate and by cytokine flow cytometry, using an in-house developed methodology.[56] This method, which does not require HLA typing and simultaneously measures HCMV-specific CD4^{+} and CD8^{+} T cells, is based on the incubation of PBMC with autologous, monocyte-derived, HCMV-infected (strain VR1814) dendritic cells, followed by determination of multiple membrane/intracellular parameters using a series of fluorochrome-conjugated mAbs.[57]

One of the aims of our research has been to verify whether pregnancy is associated with some degree of immune deficiency. A first study comparing the LPR and TNF-α production during primary HCMV infection in a group of pregnant women versus a group of nonpregnant women and men concluded that there was no difference between pregnant and nonpregnant individuals, both at the qualitative and the quantitative level of the immune response.[58] This conclusion was further confirmed by a subsequent study.[59] Although it has been reported that T-cell control of microbial infections such as M. tuberculosis as well as changes in the response at the maternofetal interface and other aspects of the immune response are clearly altered in pregnant women causing some degree of immune suppression, we had no the opportunity to detect a significant impairment of anti-HCMV immune response in pregnancy. However, both of these studies as well as other reports[54,57] documented a significant association between a delayed LPR and HCMV transmission to the fetus. Although HCMV-specific IFN-γ producing T cells are detected a few days after onset of infection and act as early effectors with low proliferative activity,[60] memory T cells with high LPR and polyfunctional capacity appear in peripheral blood only several months after onset of infection.[61]

Second, it is well known that different T-cell subpopulations can be recognized through the analysis of the surface expression of chemokine receptor CCR7 (enabling T-cell migration to the lymph nodes) and the different isoforms of the tyrosine phosphatase CD45.[62] Naïve T cells (CD45RA^{+}CCR7^{+}), after their first contact with the antigen, switch from the CD45RA isoform to CD45RO, whereas memory T cells, according to their differential CCR7 expression, can be divided into (i) central memory T cells (T_{CM} cells: CD45RA^{-}CCR7^{+}), which are able to migrate to lymph nodes and possess a high proliferative potential, and (ii) effector memory T cells (T_{EM} cells: CD45RA^{-}CCR7^{-}), which have effector functions in peripheral tissues. It has since been reported that a proportion of T_{EM} cells specific for latent viruses such as HCMV can revert to the RA isoform of CD45 (T_{EMRA} cells: CD45RA^{+}CCR7^{-}) after the acute phase of infection.[63,64] Actually, in a first study, we found that a higher percentage of HCMV-specific CD4^{+} T_{EMRA} T cells was associated with a lower

chance of transmitting HCMV infection to the fetus.[65] In a very recent study, the percentage of IFN-γ producing $CD4^+$ and $CD8^+$ T cells showing a terminally differentiated effector memory phenotype ($CD45RA^+$) increased in primary HCMV infections up to 2 years pi, to levels significantly higher than those detected in remote infections.[57] In the same study, when a large number of T and NT pregnant women were examined, the percentages of both HCMV-specific IFN-γ^+ $CD4^+$ and $CD8^+$ $CD45RA^+$ T_{EMRA} T cells were significantly higher in NT women at multiple time points examined, or when significance was not reached, the *P* value was close to significance (Figure 3).[57] In addition, the comparison between NT and T women showed at 30 days pi in NT women that there was a significantly higher interleukin (IL)-2 production by HCMV-specific $CD4^+$ T cells, which did not seem to involve later time points.

Thus, the development of a T-cell response to primary HCMV infection appears to be crucial for the control of HCMV vertical transmission, and, according to studies by our group, the delayed expression of at least 3 immunological parameters, ie, LPR, CD45RA reexpression in both $CD4^+$ and $CD8^+$ HCMV-specific T cells, and IL-2 production by HCMV-specific $CD4^+$ T cells, is significantly associated with vertical transmission. However, at the moment, for prognostic purposes, none of these parameters may be considered useful on an individual basis because of the great variation in individual values detected in T and NT women. To overcome the individual immune response variability, a new strategy has recently been proposed which is based on the ratio between IFN-γ produced by HCMV-specific T cells and the nonspecific mitogen response. This ratio has been termed IFN-γ relative response (RR) and reflects the specific immune system activation against HCMV.[66] In a subgroup of women with an IFN-γ RR <1.8%, the probability of HCMV transmission was reduced from 40% to 8%. A possible explanation for this finding may be that a higher IFN-γ RR, indicating stronger activation of the immune system, is observed in women with less efficient viral clearance, leading to higher risk of HCMV transmission.

4.1 | HCMV T-cell protein specificity and vertical transmission to the fetus

A dissection of the T-cell response to 4 major HCMV proteins (immediate-early [IE] and 3 structural proteins: pp65, early-late internal tegument protein; gH/gL/pUL128L, late surface envelope gC; and gB, another late envelope glycoprotein) was conducted by using overlapping peptides (15mers) spanning the entire above-mentioned proteins and libraries of amplified (with the addition of PHA and IL-2) T cells.[67] After incubation of the amplified T-cell libraries with single protein peptide pools, T cells specific for selected antigens were measured in

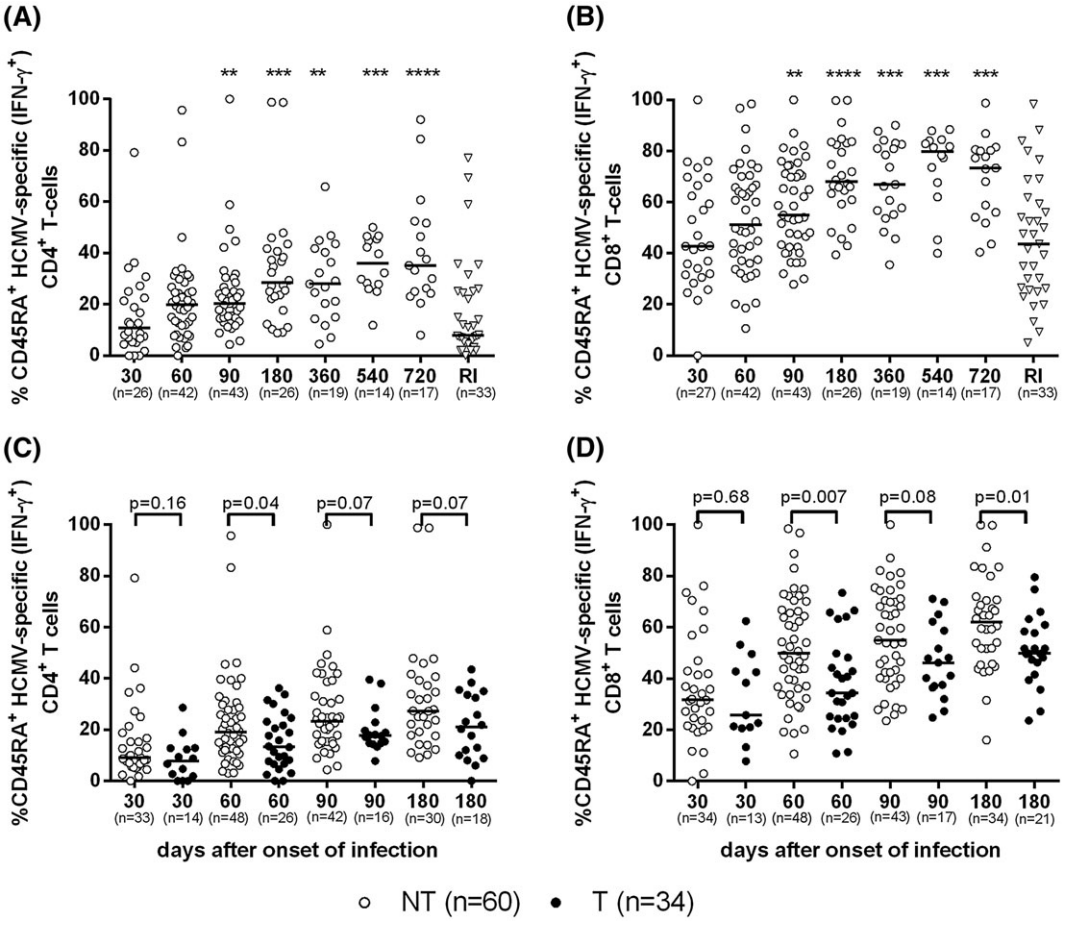

FIGURE 3 The percentages of HCMV-specific A, $CD4^+$ and B, $CD8^+$ $CD45RA^+$ T cells increased during the first 6 months after onset of primary infection (PI), reaching levels significantly higher than remote infections (RI), until 2 years pi. C-D, HCMV transmission to the fetus: starting 60 days pi, the percentages of both HCMV-specific (C) $CD4^+$ and (D) $CD8^+$ $CD45RA^+$ T cells were significantly higher or close to significance in nontransmitting (NT) as compared with transmitting (T) mothers. (From C. Fornara et al., *J Med Virol* 2016; 88: 1238-1246)

13 patients at early (1 month) and late (6-12 months) stages of primary HCMV infection. Preliminary results showed that IE is efficiently recognized by $CD8^+$ T cells, whereas gH/gL/pUL128L and gB are more efficiently recognized by $CD4^+$ T cells, and pp65 is equally recognized by $CD4^+$ and $CD8^+$ T cells (Figure 4, manuscript submitted). In addition, pp65-specific $CD4^+$ T cells and pp65- and gB-specific $CD8^+$ T cells seem to decrease at late stages of infection. However, when a group of T women were compared with a group of NT women, the antigen-specific T-cell frequency did not appear to show any significant difference with respect to transmission. Thus, no association between T-cell antigen specificity (among specificities examined) and transmission of HCMV to the fetus was detected.

4.2 | IL-7 receptor (R)^{neg} versus IL-7R^{pos} $CD4^+$ and $CD8^+$ T-cell subsets

Two IL-7R subsets for $CD4^+$ and $CD8^+$ T cells have been identified: IL-7R^{neg} short-term effector with antigen-driven persistence and IL-7R^{pos} long-term memory with antigen-independent persistence.[68,69] Among total HCMV-specific $CD4^+$ and $CD8^+$ memory T cells, a higher percentage of IL-7R^{neg} $CD4^+$ and $CD8^+$ T cells was detected during primary infection, with a decrease occurring in remote (>5 years) infections. A comparison showed a significantly higher percentage of IL-7R^{neg} HCMV-specific $CD4^+$ T cells in both early and late stages of infection in women T the infection to the fetus (Figure 5, manuscript submitted). By contrast, no difference was observed for the percentage of IL-7R^{neg} HCMV-specific $CD8^+$ T cells between T and NT mothers. We can speculate that a prompt control of HCMV infection and rapid virus clearance could be the basis of the presence of lower percentage of IL-7R^{neg} HCMV-specific $CD4^+$ T cells observed in NT mothers. In line with this hypothesis, in kidney transplant recipients, the percentage of IL-7R^{neg} HCMV-specific $CD8^+$ T cells was shown to correlate with peak viral load in the acute phase of infection,[69] and it was suggested that the strength of T-cell stimulation determines IL-7 responsiveness.[70] Thus, it is conceivable that a lower percentage of IL-7R^{neg} and a higher percentage of IL-7R^{pos} $CD4^+$ T cells is the consequence of a better virus control (and, thus, lower antigen load and T-cell stimulation). The more rapid virus clearance may be induced by either the first line of innate immune defense or by a prompt generation of adaptive immunity, and would lead to a lower risk of virus transmission to the fetus.

5 | PREVENTION OF PRIMARY HCMV INFECTION BY IMMUNOTHERAPY

To date, 2 approaches have been proposed for prevention of primary HCMV infection in HCMV-seronegative women: passive immunotherapy (Ig preparation administration) and active immunotherapy (vaccination). In addition, while we are waiting for an HCMV vaccine to become available, the adoption of hygiene measures in seronegative pregnant women appears to be the simplest as well as the most efficacious method.[71]

5.1 | Passive immunotherapy

In 2005, a nonrandomized study claimed that the administration of HCMV-specific hyperimmune globulin to pregnant women with primary infection significantly reduced the rate of mother-to-fetus transmission from 40% to 16%, whereas the risk of congenital disease also decreased significantly from 50% to 3%.[72] Subsequently, other nonrandomized studies reported a decrease in the number of congenitally infected babies born to mothers treated with hyperimmune globulin, or improved outcomes in HCMV-infected infants.[6,73–76] However, a randomized controlled clinical trial, powered by the results of the first study, did not show a significant reduction in the rate of HCMV transmission among women receiving hyperimmune globulin as compared with women receiving placebo.[7] Nonetheless, because

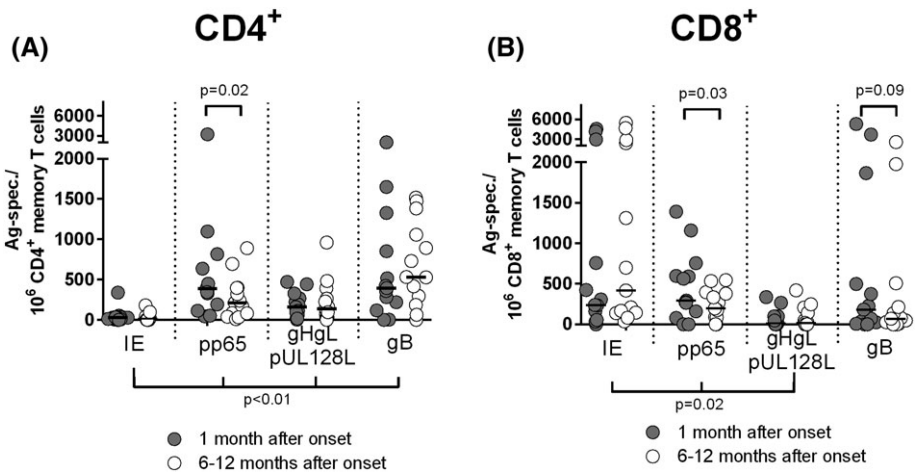

FIGURE 4 Frequency of A, $CD4^+$ and B, $CD8^+$ memory T cells specific for HCMV proteins IE-1, pp65, gHgLpUL128L, and gB in 13 pregnant women tested within one (white dots), and 6 to 12 months (gray dots) after onset of primary HCMV infection. Each dot represents a single individual, and horizontal bars indicate median values. Statistically significant differences in the frequency of protein-specific T cells between early and late time points are reported at the top of each column pair: pp65-specific $CD4^+$ and $CD8^+$ T cells decrease significantly at the late time points after infection. Statistically significant differences in the frequency of protein-specific T cells within $CD4^+$ or $CD8^+$ T cells are reported at the panel bottom. In detail, IE-1-specific $CD4^+$ T cells are significantly less in number than pp65-, gHgLpUL128L-, and gB-specific $CD4^+$ T cells, whereas gHgLpUL128L-specific $CD8^+$ T cells are significantly less in number than IE-1- and pp65-specific $CD8^+$ T cells

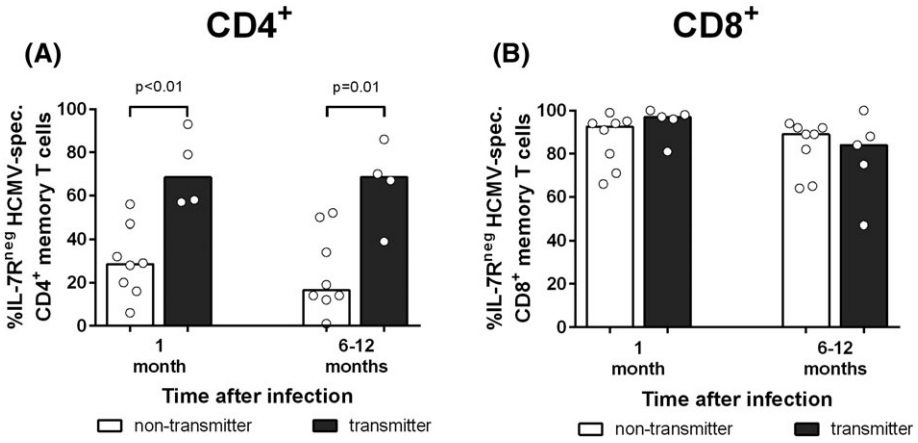

FIGURE 5 Percentage of IL-7R^{neg} T cells among HCMV-specific A CD4$^+$ or B, CD8$^+$ memory T cells in transmitting (T, n = 8) or nontransmitting pregnant women (NT, n = 4 for CD4$^+$ and n = 5 for CD8$^+$ T cells). Patients were examined 1 month and 6 to 12 months after primary infection onset. Each dot represents a single individual, and the top bars of each histogram indicate median values. Statistically significant differences between the 2 groups of T and NT women are shown: the percentage of IL-7R^{neg} cells among HCMV-specific CD4$^+$ T cells is higher in transmitting mothers at both time points examined. No difference between T and NT women is observed for IL-7R^{neg} HCMV-specific CD8$^+$ T cells

there was a 14-percentage-point difference in the rate of congenital infections between treated (30%) and control mothers (44%) with respect to the difference expected (24%), the study would have required a 3-fold greater enrollment to detect a true difference of this magnitude. This is the reason why large-scale randomized trials are still ongoing to draw conclusions on the utility of administering hyperimmune globulin to pregnant women with primary infection. At the moment, it remains entirely unresolved whether hyperimmune (or just immune) globulin administration has a role in prevention for pregnant women with nonprimary infection.

5.2 | Active immunotherapy (vaccination)

There are 2 major approaches to active immunization in HCMV-seronegative women before pregnancy: (i) one consists of a whole virus vaccine, which is attenuated in its pathogenic potential, and (ii) the other is a recombinant protein vaccine, consisting of one or more viral proteins or protein complexes. The major goal of an anti-HCMV vaccine is to induce a specific immune response similar to that elicited by natural infection.[77] Undoubtedly, natural infection confers protection against both horizontal and vertical transmission.[78–81]

However, it remains unclear why an HCMV-seropositive woman is susceptible to reinfection.[82–87] Recently, a new ELISA method based on serum reactivity with polymorphic regions of HCMV gH and gB glycoproteins has been proposed for the determination of strain-specific serological response.[88] Because of the variability of the individual immune response in association with the presence of viral mixtures and the rapid evolution of the viral population in a given host,[89,90] this new approach to diagnosis of reinfection awaits confirmation by other studies.

6 | CONCLUSION

Taken together, while waiting for an HCMV vaccine to become available, these findings suggest that the rapid control of HCMV viremia by the immune response is associated with a lower risk of virus transmission to the fetus. However, none of the parameters examined permits the discrimination of which individual mothers will transmit the virus or not.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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