Functional Exhaustion Limits CD4+ and CD8+ T-Cell Responses to Congenital Cytomegalovirus Infection

Ariane Huygens,1 Sandra Lecomte,1 Marie Tackoen,2 Véronique Olislagers,1 Yves Delmarcelle,6 Vivine Burny,1 Michel Van Rysselberge,7 Corinne Liesnard,4 Martin Larsen,7 Victor Appay,7 Catherine Donner,5 and Arnaud Marchant1,6

1Institute for Medical Immunology, Université Libre de Bruxelles, Charleroi, 2Department of Neonatology, 3Department of Obstetrics and Gynecology, Hôpital Saint-Pierre, 4Department of Virology, 5Department of Obstetrics and Gynecology, Hôpital Erasme, Brussels, and 6ImmuneHealth, Gosselies, Belgium; and 7Hôpital Pitié-Salpêtrière; Université Pierre et Marie Curie-Paris 6, France

Background. Cytomegalovirus (CMV) infection during fetal life causes severe symptoms and is associated with prolonged viral excretion. Previous studies reported low CD4+ T-cell responses to CMV infection in early life, contrasting with large responses of effector CD8+ T cells. The mechanisms underlying the defective CD4+ T-cell responses and the possible dissociation with CD8+ T-cell responses have not been clarified.

Methods. The magnitude and the quality of the fetal CD8+ and CD4+ T-cell responses to CMV infection were compared to those of adults with primary or chronic infection.

Results. In utero CMV infection induced oligoclonal expansions of fetal CD4+ and CD8+ T lymphocytes expressing a T-helper type 1 or Tc1 effector phenotype similar to that of adult CMV-specific cells. However, the effector cytokine responses and the polyfunctionality of newborn CD4+ and CD8+ T cells were markedly lower than those of adult cells. This reduced functionality was associated with a higher expression of the programmed death 1 inhibitory receptor, and blockade of this receptor increased newborn T-cell responses.

Conclusions. Functional exhaustion limits effector CD4+ and CD8+ T-lymphocyte responses to CMV during fetal life.

Keywords. cytomegalovirus; congenital infection; fetus; CD4 T cell; CD8 T cell; exhaustion.

Cytomegalovirus (CMV) is a member of the Betaherpesvirinae subfamily and establishes lifelong persistence following primary infection. Human CMV is the most common cause of congenital infection, affecting 0.2%–2% of all live births [1]. CMV infection is usually asymptomatic in immunocompetent adults, but about 20% of infected newborns develop symptoms either in utero or during the first years of life [1, 2]. In addition, both symptomatic and asymptomatic children excrete the virus for several years after birth, whereas viral excretion is usually controlled within several months in adults [3, 4]. This reduced control of CMV replication suggests a limitation in cell-mediated immune responses in early human life [5].

In adults, CMV infection induces large oligoclonal expansions of CD4+ and CD8+ T cells that express a late-differentiation phenotype characterized by the loss of expression of the costimulatory molecules CD27 and CD28 and that produce multiple antiviral cytokines [6–10]. Historical studies suggested that congenitally infected newborns have defective T-cell responses to CMV [3, 11, 12]. More-recent studies reported low or undetectable CD4+ T-cell responses to CMV antigens in infants infected in utero or after birth [11, 13, 14]. In contrast, several reports demonstrated large CD8+ T-cell responses to congenital or postnatal CMV infection [15–20]. These responses involved large expansions of effector cells expressing a late-differentiation phenotype, similarly to the adult responses [15]. As a similar dissociation between the presence of detectable CD8+ T-cell responses and very low CD4+ T-cell responses has also been observed in infants infected with
human immunodeficiency virus (HIV), it was recently proposed that the immune system in early life may have a higher capacity to develop effector CD8+ T-cell responses rather than CD4+ T-cell responses to viral pathogens [21, 22]. However, longitudinal studies of children infected in utero or soon after birth indicate that the frequency of CMV-specific CD8+ T cells producing interferon γ (IFN-γ) increases during the first year of life [18, 20]. As CMV-specific CD8+ T cells were identified in these studies on the basis of their production of cytokines, it is unclear whether the increased frequencies were related to cell multiplication or to an increased capacity of the cells to produce cytokines. Similarly, it is unclear whether the defective CD4+ T-cell responses observed following CMV or HIV infection in early life are related to a defective expansion of virus-specific cells or to a reduced capacity to produce cytokines.

In this study, we demonstrate that congenital CMV infection induces expansions of effector CD4+ and CD8+ fetal T lymphocytes. However, both CD4+ and CD8+ CMV-specific T cells had a reduced capacity to produce cytokines as compared to adult cells and expressed higher levels of the inhibitory receptor programmed death 1 (PD-1). These results provide the first evidence that functional exhaustion can limit T-cell responses to a viral infection in early life.

MATERIALS AND METHODS

Study Design

This study was approved by the ethics committees of Hôpital Erasme and Hôpital Saint-Pierre, Brussels, and Hôpital Tivoli, La Louvière. Pregnant women with primary CMV infection and their newborns were enrolled after mothers provided written informed consent. Diagnosis of primary maternal infection was performed as previously described [23]. No anti-CMV therapy was given to the mothers. Diagnosis of congenital infection was based on the detection of CMV genome by polymerase chain reaction or of CMV virus by viral culture in amniotic fluid or in newborn urine specimens collected during the first week of life. Outcome of pregnancies and clinical information on the fetuses and newborns are presented in Supplementary Table 1. Maternal blood was collected within the first 3 days after delivery. The study included 28 mothers with primary CMV infection, 26 newborns with congenital infection, and 5 uninfected newborns. Diagnosis of primary maternal infection was made at a mean gestational age (± standard deviation [SD]) of 15 ± 8 weeks. In addition, samples from 14 pregnant women with primary CMV infection and 10 infected newborns participating in the ongoing GlaxoSmithKline Biologics sponsored study (clinical trials registration NCT01251744) were analyzed in agreement with the study protocol and consent form. Diagnosis of primary maternal infection in this group was made at a mean gestational age (±SD) of 22 ± 6 weeks. Twenty-four healthy subjects chronically infected with CMV were recruited as controls by ImmuneHealth, Gosselies. Adult peripheral blood mononuclear cells (PBMCs) and cord blood mononuclear cells (CBMCs) were isolated by gradient centrifugation and were analyzed immediately or, more commonly, after storage in liquid nitrogen. Erythrocytes were depleted from CBMCs with purified anti-human CD235ab antibody (Imtec) and Dynabeads Pan Mouse immunoglobulin G (Invitrogen) according to the instructions of the manufacturer.

T-Cell Repertoire and Phenotype

The analysis of the Vβ repertoire was performed by flow cytometry using the IOTest Beta Mark TCR V Kit (Beckman Coulter) according to the instructions of the manufacturer. Cells were phenotyped with the antibodies listed in Supplementary Table 2.

T-Cell Cytokine Production and Proliferation

PBMCs and CBMCs (2 × 106 cells/mL) were cultured in Roswell Park Memorial Institute 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum (PAA) and stimulated for 6 hours with pools of 15-amino acid peptides overlapping by 11 amino acids and covering the total sequence of CMV proteins (1.5 µg/mL per peptide; JPT, Germany). Brefeldin A (BFA; 2 µg/mL; Sigma-Aldrich) was added after 1 hour of stimulation. CD8+ T cells were stained with HLA-A2 or HLA-B7 dextramer-loaded Virusys) for 7 days and a PD-1 blocking antibody or an isotype control antibody (Imtec) at 5 µg/mL. Cells were pulsed with BrdU for the last 18 hours of stimulation and were stained with the reagents listed in Supplementary Table 2. The secretion of cytokines (IFN-γ and macrophage inflammatory protein 1β [MIP-1β]) was measured in supernatants obtained on day 7, using commercially available enzyme-linked immunosorbent assays (eBiosciences).

Flow Cytometry Analysis

Interexperiment standardization of mean fluorescence intensities was performed using SPHERO Rainbow Beads (BD Biosciences, Erembodegem, Belgium). Data were obtained on a Cyan ADP LX9 cytometer (DakoCytomation) and analyzed using FlowJo 9.6 software (TreeStar, Ashland, Oregon). For functional analyses, background responses were subtracted using the software Pestle (courtesy of Mario Roederer, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes
of Health [NIH], Bethesda, Maryland) and samples with a viability <70% (Violet Live/Dead kit, Invitrogen, Molecular Probes) were excluded. Detectable responses were defined as production of at least one of the 4 cytokines by ≥0.05% or ≥0.1% of the total CD4⁺ T-cell or CD8⁺ T-cell populations, respectively. Polymorphism was analyzed with SPICE (version 5.1; courtesy of Mario Roederer and Joshua Nozzi, NIAID, NIH) and FUNKY CELLS Data Miner as previously described [24, 25].

Statistical Analysis

Data are presented as individual values, median values and interquartile ranges, or mean values and standard errors of the mean. Multiple parameter comparisons were performed with 2-way analysis of variance. When significant differences were observed, data were compared using the Mann–Whitney U test. Statistical significance was defined at P values of <0.05. GraphPad Prism 5 was used to perform the analyses.

RESULTS

Congenital CMV Infection Induces Oligoclonal Expansions of Fetal T-Helper Type 1 (Th1) and Tc1 Lymphocytes

The response of fetal CD4⁺ and CD8⁺ T lymphocytes to CMV infection was studied by comparing newborns infected in utero (CB⁺), uninfected newborns (CB⁻), their mothers who had developed primary CMV infection during pregnancy (MB), and healthy adults with chronic CMV infection (chronic). The impact of CMV infection on the differentiation of fetal CD4⁺ and CD8⁺ T lymphocytes was first analyzed by measuring the presence of cells expressing the late-differentiation phenotype (CD27⁻CD28⁻) characteristic of CMV-specific T cells (Figure 1A). As expected, CD4⁺ and CD8⁺ T lymphocytes from uninfected newborns expressed the CD27 and the CD28 molecules, in agreement with their naive phenotype. In contrast, high frequencies of differentiated CD27⁻CD28⁻ CD4⁺ T cells were detected in newborns with congenital CMV infection. These frequencies ranged from 16% to <0.05% and were significantly lower than those measured in adults with primary or chronic infection. CD4⁺ T cells expressing an intermediate differentiation phenotype (CD27⁻CD28⁻) were undetectable in most infected newborns (Figure 1A). As previously described, high frequencies of CD8⁺ T cells expressing a late (CD27⁻CD28⁻) or intermediate (CD27⁻CD28⁻) differentiation phenotype were also detected in congenitally infected newborns, and these frequencies were comparable to those for adults with primary or chronic infection (Figure 1A) [15, 19]. In agreement with their late-differentiation phenotype, CD27⁻CD28⁻ CD4⁺ and CD27⁻CD28⁻ CD8⁺ T lymphocytes from infected newborns expressed an effector phenotype characterized by decreased expression of CCR7 and IL-7R and increased expression of CD57 as compared to naive T cells (Figure 1B). In addition, newborn differentiated CD4⁺ and CD8⁺ T cells expressed high levels of the transcription factor T-bet and the chemokine receptor CCR5, indicating Th1 and Tc1 phenotypes, respectively (Figure 1B). Th1 CD4⁺ and Tc1 CD8⁺ cells are T-cell subsets producing antiviral cytokines and specialized in the control of intracellular pathogens. The phenotype of the differentiated newborn T cells was identical to that observed in adults with primary CMV infection (Figure 1B). The impact of congenital CMV infection on the repertoire of fetal T cells was assessed by measuring the frequencies of T-cell receptor (TCR) Vβ families within the CD4⁺ and CD8⁺ T-cell subsets (Figure 2). As expected, similar frequencies of the different Vβ families were detected in naive T-cell populations. In contrast, expansions at specific Vβ families were detected within differentiated CD4⁺ and CD8⁺ T-cell lymphocytes and involved different Vβ families in CD4⁺ and CD8⁺ T-cell subsets. Together, these results indicate that congenital CMV infection induces oligoclonal expansions of fetal Th1 and Tc1 effector T lymphocytes. Oligoclonal expansions are referred to as expansions of a limited set of T-cell clones, suggesting an antigen-specific response, rather than a nonspecific polyclonal T-cell activation.

Fetal CD4⁺ and CD8⁺ T Lymphocytes Have Low and Paucifunctional Cytokine Responses to CMV Antigens

The functional response of fetal CD4⁺ and CD8⁺ T cells was measured by intracellular staining of cytokines (IFN-γ, MIP-1β, tumor necrosis factor α [TNF-α], and interleukin 2 [IL-2]) following stimulation with a panel of pools of overlapping peptides derived from 10 immunodominant and subdominant CMV proteins [26]. The proportion of antigens eliciting a detectable response to individual antigens was calculated for each subject (Figure 3A). Most newborns had no detectable CD4⁺ T-cell response to CMV antigens (Figure 3A). Importantly, very low or undetectable responses were also observed in newborns with the highest frequencies of differentiated CD27⁻CD28⁻ CD4⁺ T cells and cytokine-producing cells consistently expressed a late-differentiation phenotype characterized by the downregulation of CD28 (Figure 3B). In contrast, high frequencies of CD4⁺ T cells responding to at least 1 CMV antigen were detected in adults with primary or chronic CMV infection (Figure 3A). Of note, CD4⁺ T cells from adults with primary infection responded to fewer antigens and had lower frequencies of cells responding to pp65, IE1, and UL82 antigens than adults with chronic infection (Figure 3A). Newborn CD8⁺ T cells also responded to fewer antigens than adult cells, and the frequencies of cells responding to IE1 or UL55 were lower than in adults with primary CMV infection (Figure 3A). On the other hand, lower frequencies of CD8⁺ T cells responding to UL32 or UL55 antigens were detected during primary infection as compared to chronic infection (Figure 3A). These results indicate that the breadth of CMV antigens inducing detectable CD4⁺ and CD8⁺ T-cell responses was lower in CMV-infected newborns than in adults and that
Figure 1. Congenital cytomegalovirus (CMV) infection induces the differentiation of fetal T-helper type 1 and Tc1 cells. Cord blood mononuclear cells and peripheral blood mononuclear cells were obtained from CMV-uninfected newborns (CBni) and CMV-infected newborns (CBi), from their mothers with primary CMV infection (MB), and from chronically infected adults (chronic). A, The percentage of CD4+ or CD8+ T cells expressing an intermediate (CD27–CD28+ or CD27+CD28−, respectively) or late (CD27–CD28−) differentiation phenotype within the total CD4+ or CD8+ T-lymphocyte populations was measured by flow cytometry. A representative example is shown for each study group. Data are median values for 6 CBni, 2 CBi, 23 MB, and 11 chronic subjects. B, The expression of CCR7, interleukin 7 receptor (IL-7R), CD57, T-bet, and CCR5 by differentiated (CD27–CD28−; gray histograms) and naive (CD45RO−CD27+/CD28+; white histograms) CD4+ and CD8+ T cells from CBi and MB was measured by flow cytometry. *P < .05, **P < .01, and ***P < .001. Abbreviation: FMO, fluorescence minus one.
similar differences, but of lower magnitude, were observed between adults with primary or chronic infection.

To gain insight into the functionality of fetal CMV-specific CD4+ and CD8+ T cells, their capacity to produce multiple cytokines (polyfunctionality) was analyzed among the detectable responses (see “Materials and Methods” section). Newborn CMV-specific CD4+ and CD8+ T cells included a lower proportion of cells producing multiple cytokines as compared to adults with primary or chronic infection (Figure 4A). Most newborn cells produced only 1 or 2 cytokines, whereas most adult cells produced 2 or 3 cytokines. MIP-1β was the most commonly produced cytokine, whereas IL-2 was the least commonly produced cytokine. Similar results were obtained with total CMV-specific CD8+ T cells (Figure 4A). These results were further confirmed by the analysis of CMV dextramer+ CD8+ T cells. As shown in Figure 4B, newborn CMV dextramer+ CD8+ T cells included lower frequencies of cytokine-producing cells and were less polyfunctional than adult cells. Of note, CMV dextramer+ CD8+ T cells were mostly CD28- in newborns and adults, further supporting the notion that during primary infection, CMV-specific T cells express a late-differentiated phenotype in fetuses and in adults [27]. Cell polyfunctionality was then quantified using a previously described index [25]. Newborn CMV-specific CD4+ and CD8+ T cells had a lower polyfunctionality index than cells from adults with primary or chronic CMV infection (Figure 4A and 4B). The polyfunctionality index of total CMV-specific CD8+ T cells was also lower in adults with primary infection as compared to those with chronic infection. Together, these results indicate that the magnitude and the polyfunctionality of newborn CD4+ and CD8+ T-cell responses to CMV are lower than in adults.

CD4+ and CD8+ T Lymphocytes Induced by Congenital CMV Infection Express High Levels of PD-1

The reduced polyfunctionality of newborn CD4+ and CD8+ T cells is analogous to the functional profile of exhausted T lymphocytes observed during persistent viral infections. Exhausted T lymphocytes have a reduced capacity to produce cytokines and express high levels of inhibitory receptors, of which PD-1 is the most characteristic [28]. Compared with naive T cells, differentiated CD4+ and CD8+ T cells and CMV dextramer+ CD8+ T cells expressed higher levels of PD-1 in the 3 study groups. Differentiated newborn CD4+ and CD8+ T cells expressed higher levels of PD-1 than adult T cells (Figure 5A–C). As shown in Figure 5D and 5E, CMV antigens induced only low or undetectable proliferative responses or cytokine secretion in T cells from CMV-infected newborns. Blocking PD-1 upregulated these responses, indicating that the inhibitory receptor was functional and controlled newborn T-cell responses to CMV antigens.

DISCUSSION

This study demonstrates that congenital CMV infection induces the differentiation and functional exhaustion of fetal effector Th1 and Tc1 cells. High frequencies of both CD4+ and CD8+
T cells expressing a late-differentiation effector phenotype were detected in infected newborns. Differentiated newborn T cells expressed high levels of the Th1/Tc1 transcription factor T-bet and the chemokine receptor CCR5. This phenotype is identical to the one expressed by adult CMV-specific T cells during primary or chronic infection [29–31].

Figure 3. Newborn CD4+ and CD8+ T lymphocytes have low cytokine responses to cytomegalovirus (CMV) antigens (Ags). Cord blood mononuclear cells and peripheral blood mononuclear cells were obtained from CMV-infected newborns (CBi; n = 13–14), from their mothers with primary CMV infection (MB; n = 12–13), and from chronically infected adults (chronic; n = 14) and were stimulated with a panel of peptide pools from immunodominant and subdominant CMV Ags. A, The percentage of CD4+ and CD8+ T lymphocytes producing any cytokine (interferon γ [IFN-γ], macrophage inflammatory protein 1β [MIP-1β], tumor necrosis factor α [TNF-α], and interleukin 2 [IL-2]) was measured by intracytoplasmic staining and flow cytometry. The proportion of Ags recognized by CD4+ and CD8+ T cells from each subject in the 3 study groups is shown in the left panel. Bars denote median values. Individual responses of CD4+ and CD8+ T cells producing any cytokine to each CMV Ag is shown on the right panel. Significant differences between CBi and MB (*) and between MB and chronic subjects (°) are shown. *,°P < .05, **,°°P < .01, and ***,°°°P < .001. Bars denote median values. B, Representative example of flow cytometry dot plots showing cytokine staining within unstimulated (upper panel) and CMV antigen (US3)–stimulated (lower panel) newborn CD4+ T cells. Numbers in quadrants indicate percentages of CD4+ T cells.
Figure 4. Newborn CD4+ and CD8+ T lymphocytes have paucifunctional cytokine responses to cytomegalovirus (CMV) antigens. A, The polyfunctionality of CMV-specific CD4+ and CD8+ T cells producing at least one of the 4 cytokines (interferon γ [IFN-γ], macrophage inflammatory protein 1β [MIP-1β], tumor necrosis factor α [TNF-α], and interleukin 2 [IL-2]) in response to any pool of peptides in CMV-infected newborns (CB1), their mothers (MB), and healthy adults with chronic CMV infection (chronic) was analyzed using SPICE software (left panel). The median proportion of CD4+ and CD8+ T cells producing 1, 2, 3, or 4 cytokines is shown for each study group. Nonresponders were excluded from the analysis, as defined in “Materials and Methods”. The polyfunctionality index (PI) of the cells was calculated using FUNKY CELLS software (right panel). B, The same analysis was performed on CMV dextramer+ CD8+ T cells, except that the total population of dextramer+ cells, including cells producing no detectable cytokine (0 cytokine), was included in the analysis. Bars denote median values. *P < .05, **P < .01, and ***P < .001. The upper part of Figure 4B presents a representative example of flow cytometry dot plots showing the production of IFN-γ by and the phenotype (expression of the differentiation markers CD28 and CD45RO) of tetramer+ CD8+ T cells. Numbers in quadrants indicate percentages of tetramer+ CD8+ T cells.
CD4+ and CD8+ T lymphocytes from cytomegalovirus (CMV)-infected newborns express high levels of programmed death 1 (PD-1). A–C, The expression of PD-1 by naive and differentiated CD4+ T cells (A), CD8+ T cells (B), and CMV dextramer+ CD8+ T cells (C) from infected newborns (CB; n = 8) and of adults with primary infection (MB; n = 12) or chronic infection (Ch; n = 10–11) was measured by flow cytometry. Representative dot plots of cells from CMV-infected newborns are shown in the left panels. Shown are individual data and median values. D and E, The proliferative responses of CD4+ and CD8+ T cells (D) and the secretion of cytokines (E) were measured in CMV-infected newborns after 7 days of stimulation with CMV Ags. Proliferation was measured using the BrdU incorporation assay, and cytokine secretion was measured by enzyme-linked immunosorbent assay. Data are median values ± interquartile ranges for 10 subjects. *P < .05, **P < .01, and ***P < .001. Abbreviations: FS, forward scatter; IFN-γ, interferon γ; MIF, mean fluorescence intensity; MIP-1β, macrophage inflammatory protein 1β.
newborn CD4+ and CD8+ T-cell populations were enriched in expansions involving specific TCR Vβ families, supporting the notion that the responses are antigen specific and not the result of a bystander activation. This notion was further supported by the observation that most newborn CD4+ T cells producing cytokines in response to CMV antigens and that CMV-tetramer+ newborn CD8+ T cells expressed a late-differentiation phenotype.

Previous studies reported low or undetectable responses of CD4+ T cells to congenital CMV infection, and it has been proposed that the fetal immune system may be particularly unable to develop antiviral Th1 responses [22]. Supporting this possibility, several factors have been described that could limit the differentiation of Th1 cells in early life, including a reduced production of IL-12 by dendritic cells and a hypermethylation of the promoter in the gene encoding IFN-γ [32–35]. Our observations indicate that these factors do not prevent the differentiation of Th1 cells following CMV infection. However, cytokine responses to CMV antigens were markedly lower in newborns than in adults with primary CMV infection. Importantly, this lower antigen loads inducing the loss of IL-2 production and higher antigen loads progressively inducing the loss of TNF-α, IFN-γ, and finally MIP-1β production [28]. This hierarchy of cytokine production is the same as the one observed in CMV-infected newborns, further supporting the notion that fetal CD4+ and CD8+ effector T cells are functionally exhausted. PD-1 blockade increased the proliferative and cytokine responses of newborn cells after 7 days of antigen stimulation but did not significantly influence cytokine responses after short-term stimulation (data not shown). Similar differences in the impact of PD-1 blockade on short-term and long-term in vitro stimulation were observed in adults with chronic viral infections and indicate that factors other than PD-1 contribute to the functional exhaustion of newborn cells [37, 39]. Adult T cells also had reduced polyfunctionality during primary compared to chronic CMV infection. Together, these data indicate that primary CMV infection induces the functional exhaustion of both fetal and adult T cells and that the level of exhaustion is more pronounced in the fetus. As the level of T-cell exhaustion has been linked to the strength of T-cell stimulation [28], this difference may be related to the exposure of fetal T cells to higher CMV antigen loads than adult cells. Supporting this possibility, higher viral loads have been reported in CMV-infected fetuses as compared to their mother with primary infection [40–42]. Such difference may be related to a lower control of CMV replication by the fetal immune system but may also involve the ingestion of high CMV loads excreted in the amniotic fluid [41, 42]. Alternatively, the quality of the signals provided by fetal antigen-presenting cells may favor the emergence of functionally regulated T cells in utero, as observed in animal models of adaptive tolerance [43]. Finally, fetal T cells are programmed differently than adult cells and may therefore be intrinsically more susceptible to functional exhaustion [44].

The functional exhaustion of newborn T cells is likely to reduce their capacity to control viral replication and may therefore play an important role in the prolonged viral excretion associated with CMV infection in early life. Indeed, CMV-specific CD4+ T-cell responses increase during the first 2 years of life, and the cessation of viruria correlates with the acquisition of proliferative responses to CMV antigens [3, 45]. A similar association between functional exhaustion and intense viral excretion was recently observed following primary CMV infection of juvenile rhesus macaques [46]. Studies encoding PD-1 was recently associated with CMV infection in kidney transplanted patients [36]. To our knowledge, this is the first observation of increased PD-1 expression during a fetal immune response. Reduced polyfunctionality and high expression of PD-1 are central characteristics of functionally exhausted T cells [37, 38]. T-cell exhaustion is induced by chronic viral infections involving a prolonged exposure to high antigen loads. It is characterized by the hierarchical loss of T-cell functions, with lower antigen loads inducing the loss of IL-2 production and higher antigen loads progressively inducing the loss of TNF-α, IFN-γ, and finally MIP-1β production [28]. This hierarchy of cytokine production is the same as the one observed in CMV-infected newborns, further supporting the notion that fetal CD4+ and CD8+ effector T cells are functionally exhausted. PD-1 blockade increased the proliferative and cytokine responses of newborn cells after 7 days of antigen stimulation but did not significantly influence cytokine responses after short-term stimulation (data not shown). Similar differences in the impact of PD-1 blockade on short-term and long-term in vitro stimulation were observed in adults with chronic viral infections and indicate that factors other than PD-1 contribute to the functional exhaustion of newborn cells [37, 39]. Adult T cells also had reduced polyfunctionality during primary compared to chronic CMV infection. Together, these data indicate that primary CMV infection induces the functional exhaustion of both fetal and adult T cells and that the level of exhaustion is more pronounced in the fetus. As the level of T-cell exhaustion has been linked to the strength of T-cell stimulation [28], this difference may be related to the exposure of fetal T cells to higher CMV antigen loads than adult cells. Supporting this possibility, higher viral loads have been reported in CMV-infected fetuses as compared to their mother with primary infection [40–42]. Such difference may be related to a lower control of CMV replication by the fetal immune system but may also involve the ingestion of high CMV loads excreted in the amniotic fluid [41, 42]. Alternatively, the quality of the signals provided by fetal antigen-presenting cells may favor the emergence of functionally regulated T cells in utero, as observed in animal models of adaptive tolerance [43]. Finally, fetal T cells are programmed differently than adult cells and may therefore be intrinsically more susceptible to functional exhaustion [44].

The functional exhaustion of newborn T cells is likely to reduce their capacity to control viral replication and may therefore play an important role in the prolonged viral excretion associated with CMV infection in early life. Indeed, CMV-specific CD4+ T-cell responses increase during the first 2 years of life, and the cessation of viruria correlates with the acquisition of proliferative responses to CMV antigens [3, 45]. A similar association between functional exhaustion and intense viral excretion was recently observed following primary CMV infection of juvenile rhesus macaques [46]. Studies
suggest that functional exhaustion may also limit T-lymphocyte responses to other viral pathogens that are poorly controlled in early life, including HIV \[21, 22, 47\]. The identification of functional exhaustion as a mechanism limiting effector T-cell responses in early life has important implications for our understanding of the pathogenesis of CMV infection, as well as other chronic viral infections affecting the fetus and the young infant.

**Supplementary Data**

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

**Notes**

**Acknowledgments.** We thank all of the study participants; Dr Roland Delvaeger (Universitair Ziekenhuis Gasthuisberg, Leuven), Dr Jacques Francheotte (Centre Hospitalier Universitaire de Tivoli, La Louvière), Dr Dominique Thomas (Hôpitaux Iris Sud Ixelles, Brussels), and GlaxoSmithKline Biologicals, for providing samples from the ongoing sponsored study (NCT01251744); and Barbara Dujardin (ImmuneHealth clinical investigation unit, La Louvière), for assistance with clinical sample collection.

**Financial support.** This work was supported by the Fonds pour la Formation à la Recherche dans l’Industrie et l’Agriculture and the Fonds David et Alice van Buuren (fellowships to A. H.). A. M. is a senior research associate of the Fonds de la Recherche Scientifique (F.R.S.-FNRS), Belgium.

**Potential conflicts of interest.** GlaxoSmithKline Biologicals SA contributed to the collection of clinical samples as described in the Methods section and approved the decision to publish the findings presented here. Otherwise, funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The Institute for Medical Immunology is cofunded by the Walloon Region and GlaxoSmithKline Vaccines. A. M. has served as a consultant for GlaxoSmithKline Vaccines and Hoojika Biotech. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


Fetal T-Cell Exhaustion Following CMV Infection • JID 2015;212 (1 August) • 493


