0031-3998/04/5502-0280
PEDIATRIC RESEARCH
Vol. 55, No. 2, 2004
Printed in U.S.A.

Cellular Immune Response of Fetuses to Cytomegalovirus

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ABSTRACT

Primary infection with cytomegalovirus (CMV) in immunocompetent hosts is accompanied with activation and differentiation of naive $CD8^+$ T cells to effector/memory cells secreting interferon-γ (IFN-γ). Alteration of these responses during the perinatal period is suggested by a higher rate of CMV diseases in congenital infection. For addressing this issue, immunologic investigations were performed in 15 fetuses (22–36 wk of gestation) with documented congenital CMV infection. Results show that cellular immune responses can be detected as soon as the 22nd week of gestation (the youngest fetus analyzed). Compared with age-matched control subjects, infected fetuses evidence a dramatic increase in the percentages of activated and terminally differentiated CD8 T cells. Indeed, median percentages (interquartile range) of HLA-DR^+ and of $CD28^-CD8^+$ T cells were 24% (19–34) and 38% (24–52), respectively in infected fetuses *versus* 3% (0–4) for each subset in control subjects. In addition, the percentages of T cells secreting IFN-γ after *in vitro* stimulation with phorbol myristate acetate and ionomycin was significantly higher in infected fetuses [10% (5–25)] than in healthy fetuses [0.8% (0.6–1.2)] with IFN-γ being mostly secreted by $CD8^+$ T cells and to a lesser extend by $CD4^+$ T cells. These cellular immune responses have clear similarities with responses previously reported in adults. Cellular immunity to CMV, however, might not be fully functional in fetuses. Indeed, the number of T cells capable of secreting IFN-γ is strikingly lower after *in vitro* stimulation with the CMV-specific antigen than after *in vitro* stimulation with phorbol myristate acetate/ionomycin that bypasses signaling through the T-cell receptor. **(*Pediatr Res* 55: 280–286, 2004)**

Abbreviations

CMV, cytomegalovirus
IFN-γ, interferon-γ
PMA, phorbol myristate acetate
Ca^{2+}, ionomycin
PBMC, peripheral-blood mononuclear cells

$CD8^+$ T lymphocytes are believed to be an important host defense against viruses. This concept is supported by the fact that patients with defects in cellular immunity experience more prolonged virus shedding and present more frequent and severe illness with cytomegalovirus (CMV) (1, 2). Furthermore, viral immunity to CMV can be restored by the adoptive transfer of CMV-specific $CD8^+$ T cells in immunodeficient individuals (3).

During primary infection with CMV, $CD8^+$ T cells are activated, proliferate, and differentiate to become effector and memory lymphocytes. Effector/memory $CD8^+$ T cells possess cytotoxic activity. In addition, they become capable of releasing factors with antiviral action, such as interferon-γ (IFN-γ) (4). Activation and differentiation of $CD8^+$ T cells during viral infection are associated with changes in the cell surface phenotype. In persistent virus infections, HLA-DR expression on $CD8^+$ T cells reflects immune activation (5). Also, during the course of infection, part of CD8 T cells switch progressively to a long-lived $CD8^+$ $CD28^-$ T-cell pool (6), which contains virus-specific, cytolytic T lymphocytes (7, 8). It has been suggested that these $CD28^-$ $CD8^+$ T cells might be terminally differentiated because of their poor proliferative potential (9).

The great majority of primary CMV infections in immunocompetent hosts are clinically silent, whereas the rate of CMV disease is 10–15% in congenital infection (10). This difference is supposed to be related to the immaturity of the immune system. The concept of immaturity of $CD8^+$ T-cell functional responses to viruses has been largely documented in mouse models (11, 12) and also suggested in the human. As an example, the incidence of children who are younger than 6 mo of age and develop CD8 T-cell activities, as measured by cytotoxicity (13, 14) or IFN-γ secretion (15) assays, is low

Received January 20, 2003; accepted August 7, 2003.
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DOI: 10.1203/01.PDR.0000104150.85437.FE

compared with the one seen in adults. Also, cytolytic responses directed against the respiratory syncytial virus are more pronounced and more frequent in 6- to 24- than in 0- to 5-mo-old infants (16). There are few data about $CD8^+$ T-cell response to CMV in early gestational ages.

We explored cellular T-cell responses in fetuses with documented CMV infection. Our study primarily focused on the subset composition of the CD8 compartment and on the capacity of $CD8^+$ cells to become IFN-γ producer cells. Our results indicate that expansion of activated ($HLA\text{-}DR^+$) and terminally differentiated ($CD28^-$) $CD8^+$ T cells can be observed as soon as the 22nd week of gestation in fetuses infected with CMV. In addition, a consequent proportion of $CD8^+$ T cells gain the capacity of producing IFN-γ after *in vitro* exposure to the nonspecific and potent activators phorbol myristate acetate (PMA) and ionomycin (Ca^{2+}). The proportion of T cells secreting IFN-γ after *in vitro* exposure to the specific CMV antigen was clearly lower. On the basis of these data, the competence of the immune system to fight CMV during the intrapartum period is discussed.

METHODS

Patients and control subjects. The study was approved by the local committee of the Robert Debré perinatal department. For blood sampling by cordocentesis after delivery or before pregnancy termination and for the use of residual bloods from fetuses, oral information was given to the parents without written consent. For blood sampling from adult control subjects, selectively performed for the purpose of the study, all individuals gave written informed consent. Fifteen fetuses were included. Prenatal diagnosis of CMV infection was achieved for 11 fetuses during evaluation of pregnancies with mothers' primary CMV infection within the first trimester. Documentation of CMV infection was based on CMV isolation on MRC5 monolayers and CMV DNA detection by PCR (Light Cycle; Roche Diagnostics, Meylan, France) done on amniotic fluid. Diagnosis was achieved at birth for four fetuses and from mothers with primary CMV infection during the first trimester of pregnancy but without any fetus evaluation during pregnancy. Documentation of CMV infection, at birth, was based on CMV-IgM antibody detection (ELISA; VIDAS CMV IgM; Biomérieux, Maray l'étoile, France) and/or viral shedding (CMV isolation on MRC5 monolayer). The mean age of gestation at the time of immunologic evaluation was 29 wk (range, 22–38). In 11 cases, blood samples consisted of residual blood from cordocentesis dedicated to hematologic, biochemical, and/or virologic analysis in the fetus either during the pregnancy evaluation ($n = 5$) or during elective termination of pregnancy ($n = 6$). Of the six elective terminations of pregnancy, one was requested by the parents and five were performed because of ultrasound and/or biologic abnormalities. For four fetuses, cordocentesis was performed at birth of the neonate immediately after delivery.

Controls consisted of cord blood from full-term (38–40 wk of gestation) uncomplicated pregnancies ($n = 11$) and from elective pregnancy termination for fetal malformation ($n = 43$; 17–38 wk of gestation). We also used, in some assays (Fig. 1), blood from adult volunteer donors ($n = 10$) as controls.

Blood samples were collected on EDTA (immunophenotyping assays) and on acid citrate dextrose (IFN-γ expression assays) to prevent coagulation. All tests were performed on the day of sampling.

Immunophenotyping. Direct immunofluorescence staining was performed on whole blood using a combination of two (CD4/CD45R0), three (CD3/CD8/HLA-DR, CD3/CD8/CD28, CD3/CD8/CD45RA, CD4/CD45RA/CD621), or four (CD3/CD4/CD8/CD45, CD3/CD19/CD16+CD56/CD45) directly labeled MAb. MAb were purchased from *1*) Becton-Dickinson, le Pont de Claix, France (CD4-FITC, CD45RO-PE, HLA-DR-PE, CD28-PE, CD62 L-FITC, and also CD3-FITC/CD4-APC/CD8-PE/CD45-per–CP and CD3-FITC/CD19-APC/CD16+CD56-PE/CD45-Per–CP combinations), *2*) Immunotech, Marseille, France (CD3-FITC), and *3*) Coulter, Miami, Florida, U.S.A. ($CD8^-$ PECy5). In brief, 100 μL of whole blood was added to a premixed solution of MAb at the appropriate dilution. After a 15-min incubation at room temperature in the dark, red blood cells were lysed by using FACS Lysing (Becton-Dickinson, Le Pont declai, France). Samples were washed twice and resuspended in PBS. Analysis was performed in a FACScalibur (Becton-Dickinson) instrument with the Cellquest software. For each sample, peripheral-blood mononuclear cells (PBMC) were gated into the lymphocyte population according to forward and side scatter characteristics, and 10,000 events were acquired in this gate. The absolute number of lymphocytes B, T, CD4, CD8, and NK cells was determined by using counting beads in the multitest (CD3/CD4/CD8/CD45 and CD3/CD19/CD16+CD56/CD45) flow-cytometry assays and according to the manufacturer recommendations (Becton-Dickinson).

IFN-γ expression. PBMC were isolated by differential centrifugation over "lymphocytes separation medium" (Eurobio, Les Ulis, France), and 10^6 cells/mL were resuspended in complete RPMI medium 1640 (Eurobio) supplemented with penicillin/streptomycin/L-glutamine and 10% FCS (Valbiothech, Paris, France) in culture tubes (1 mL/tube). PBMC were stimulated with ionomycin, 500 ng/mL (Calbiochem-Merck-Eurolab, Fontenay sous Bois, France), and PMA 50 ng/mL (ICN Biomedicals, Orsay, France) or a CMV antigen preparation from a commercially prepared lysate of CMV-infected fibroblasts at 10 ng/mL (complement fixation reagent; Bio Whittaker, Verviers, Belgium). Cells without stimulator were cultured in parallel to be used as negative control. Brefeldin A, 15 μg/mL (ICN Biomedicals) was added, since the second hour of culture in all tubes for intracellular accumulation of the secreted proteins. Cultures were incubated for 18 h at 37°C in a humidified CO_2 incubator before intracellular staining. After an initial wash in FACS-Flow (Becton-Dickinson), stimulated and unstimulated (negative control) cells were fixed and permeabilized according to the manufacturer recommendations (DAKO intrastain; Glostrup, Denmark). Cells were then stained with CD3-FITC (Immunotech), CD8-PECy5 (Immunotech), and anti–IFN-γ–PE (Becton-Dickinson) MAb for 20 min at room temperature in the dark. Cells were then washed twice and analyzed in a FACScalibur (Becton-Dickinson) in-

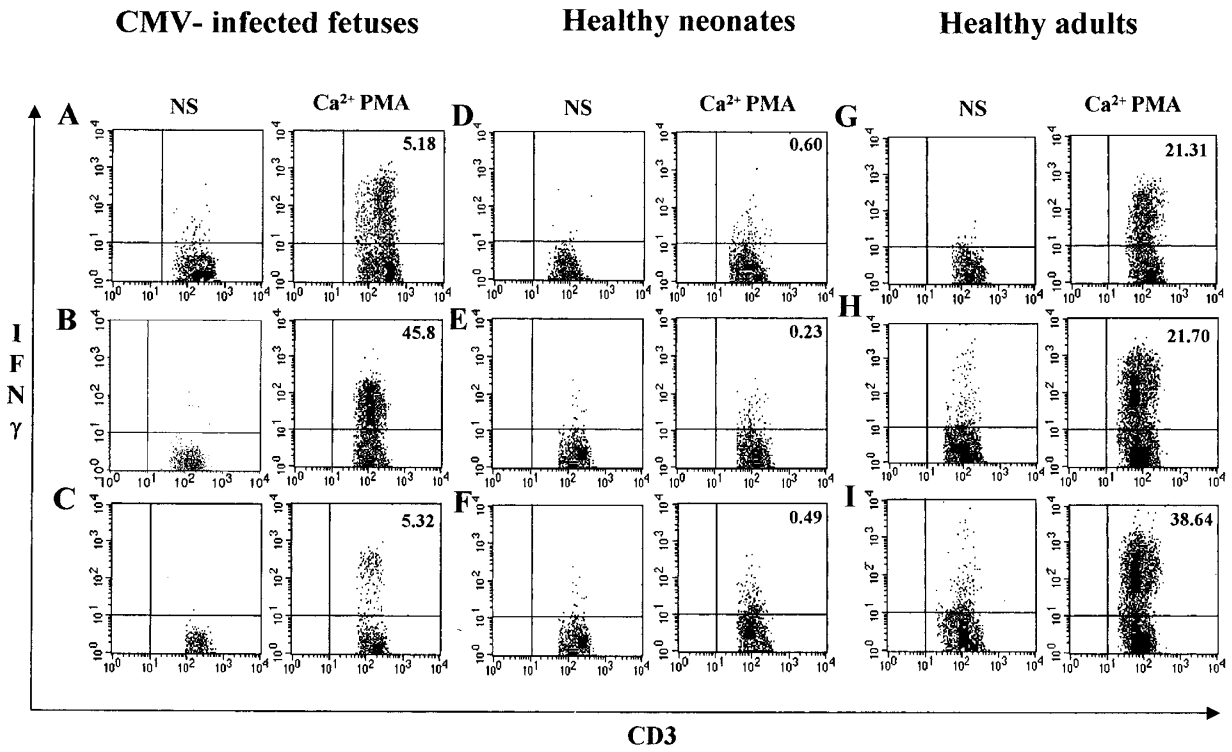

Figure 1. IFN-γ responses to Ca^{2+}/PMA by T cells from CMV-infected fetuses, healthy neonates, and healthy adults. After stimulation of PBMC with the nonspecific activator Ca^{2+}/PMA, cells were permeabilized and stained with MAb specific for IFN-γ, CD3, and CD8. We analyzed the frequency of IFN-γ–producing T cells by sequential gating on the lymphocyte population and then on $CD3^+$ lymphocytes as described in "Methods." The fraction of secreting T cells was then determined by calculating the ratio of cells expressing CD3 (*x* axis) and intracellular IFN-γ (*y* axis) over the total $CD3^+$ cells × 100. Percentages are given in the right upper quadrant. In all cases, percentages observed in nonstimulated culture (NS) are deduced. The results for three independent assays performed in three distinct fetuses infected with CMV and distinct controls are provided.

strument with the Cellquest software. Between 1 and 2.5×10^5 events were acquired depending on sample availability. Unstimulated control samples showed a low background staining that was subtracted in calculating the frequency of IFN-γ–producing cells. Staining with isotype control MAb was consistently negative. In all assays, we analyzed cells using a $CD3^+$ cell gate, and we considered $CD8^-$ cells within this gate as $CD4^+$ T lymphocytes.

Statistical analysis. Statistical analysis used a nonparametric test (Mann-Whitney) for quantitative variable. $P < 0.05$ was considered as significant.

RESULTS

Reference values for lymphocyte subsets in cord blood. At first, reference values for lymphocyte subsets were determined in a population of 54 control fetuses, from 17 to 40 wk of gestation age, either healthy or with diseases that do not alter immune system development (see "Methods"). Results showed that fetal lymphocyte absolute values increase with time from 17 to 40 wk of gestation, whereas percentages of lymphocyte subsets do not (data not shown). The medians found in the 54 fetuses (irrespective of their gestation age) therefore were used as reference values. As shown in Table 1, these reference values evidence a dominance of naive ($CD4/RA^+$ or CD8/CD45 RA^+) over memory ($CD4/CD45RO^+$ or CD8/$CD45RA^-$ cells). Very few $CD8^+$ T cells express the activation marker HLA-DR ($CD8/CD3/HLA\text{-}DR^+$). Moreover, the percentage of $CD8^+$ T cells lacking the CD28 molecule (CD8/$CD3/CD28^-$) is very low. This profile is characteristic of naive individuals who still have not experienced antigen. Of note, virtually all CD4/CD45 RA^+ cells express the selectin CD62 L (data not shown).

Preferential expansion of $CD8^+$ T cells and increased proportion of memory T cells in fetuses infected with CMV. The phenotype of circulating lymphocyte subsets was assessed in 15 CMV-infected fetuses (mean gestational age, 29 wk; range, 22–38 wk). The median of percentages found in CMV-infected fetuses was compared with the median of percentages found in the 54 control fetuses (mean gestational age, 29 wk; range, 17–40 wk). As shown in Table 1, there is no significant difference between the two groups in either total lymphocyte counts, or B lymphocyte percentages or natural killer cell percentages compared with reference values. By contrast, CMV-infected fetuses have lower $CD4^+$ (both percentages and absolute counts) and higher $CD8^+$ percentages (Table 1). In addition, a significant switch of naive ($CD45RA^+$) to memory ($CD45RA^-$ or $CD45RO^+$) phenotype is observed in patients. A significant proportion of naive $CD4/CD45RA^+$ cells have switched to the memory $CD4/CD45RO^+$ phenotype. The percentage of $CD8^+$ naive cells ($CD3/CD8/CD45\ RA^+$), decreased and the percentage of $CD8^+$ memory cells ($CD3/CD8/CD45RA^-$) increased. Of note, the CD62L molecule remained expressed on the great majority of $CD4/CD45RA^+$ cells (data not shown). Thus, CMV infection is associated with a prefer-

Table 1. *Lymphocyte subpopulations in CMV-infected fetuses*

Cell type	CMV-infected fetuses ($n = 15$)	Reference values ($n = 54$)	p values
Total lymphocytes (10^{-9}/L)*	2.7 [2.3–4.4]	2.7 [1.8–4]	NS
B lymphocytes (%)†	28 [14–40]*	19 [14–27]	NS
NK cells (%)†	06 [04–13]	07 [04–12]	NS
T lymphocytes (%)†	58 [48–62]	68 [60–75]	0.0004
CD4 (%)	34 [25–40]	49 [42–54]	0.0009
(10^{-9}/L)	0.95 [0.67–1.22]	1.33 [0.89–1.7]	0.047
CD8 (%)	24 [18–30]	20 [16–23]	0.044
(10^{-9}/L)	0.58 [0.45–1.3]	0.46 [0.37–0.71]	NS
Naive/memory cells (%)			
CD4/CD45RA$^+$ in CD4	85 [66–90]‡	94 [90–96]	0.01
CD4/CD45RO$^+$ in CD4	11 [10–20]‡	07 [05–09]	0.004
CD3/CD8/CD45RA$^+$ in CD8	92 [69–94]‡	95 [92–97]	0.026
CD3/CD8/CD45RA$^-$ in CD8	08 [06–31]‡	05 [03–08]	0.01
Activated/effector CD8 (%)			
CD3/CD8/HLA-DR$^+$ in CD8	24 [19–34]	03 [00–04]	<0.0001
CD3/CD8/CD28$^-$ in CD8	38 [25–52]	03 [00–04]	<0.0001

* Results are given as median [interquartile range].

† B lymphocytes are CD19$^+$ cells; T lymphocytes are CD3$^+$ cells; and NK are CD16$^+$/CD56$^+$/CD3$^-$ cells. $P > 0.05$ is considered nonsignificant (NS). Statistical analysis used a nonparametric test (Mann-Whitney).

‡ Seven to 12 instead of 15 infected fetuses were analyzed.

ential expansion of the CD8$^+$ over the CD4$^+$ T-cell compartment, and a switch of naive to memory T cells has been noticed.

Signs of immune activation and accumulation of terminally differentiated CD8$^+$ T cells in fetuses infected with CMV. When comparing the CD8 subsets between CMV-infected fetuses and reference values, activated (HLA-DR$^+$) and terminally differentiated (CD28$^-$) CD8$^+$ T cells differed strikingly between the two groups (Table 1). The percentage of activated CD8$^+$ T cells within the CD8$^+$ T-cell compartment (CD3/CD8/HLA-DR$^+$ in CD8) increased significantly in patients. Also, a huge increase of the CD8/CD3/CD28$^-$ subset within the CD8$^+$ T cell compartment was observed.

Because all fetuses who were infected with CMV were from mothers with documented primary infection during pregnancy, we controlled that accumulation of activated (HLA-DR$^+$) and terminally differentiated (CD28$^-$) CD8$^+$ T cells found in CMV-infected fetuses is not related to the virologic status of the mother. This was achieved by testing blood samples of neonates who were born to mothers with primary infection during pregnancy but who themselves were not infected. As expected, no change in the percentages of activated and terminally differentiated CD8$^+$ T cells was observed in fetuses when the virus was not transmitted (data not shown).

Quantification of cells producing IFN-γ after stimulation by Ca^{2+}/PMA. A cytometry assay was used to quantify T cells (CD3$^+$) capable of producing IFN-γ in response to the nonspecific Ca^{2+}/PMA polyclonal activator. It is well known that T cells fail to produce IFN-γ unless they have been primed *in vivo* by antigen. We therefore used in each assay two control populations. Cord blood from healthy neonates, that have encountered few if any antigens, were used as low-producer control subjects, and blood from healthy adults, that have been primed by numerous antigens, were used as high-producer control subjects. Results from three distinct fetuses compared with three distinct adults and three distinct healthy neonates are shown in Figure 1. They clearly show that poor IFN-γ production by T cells from healthy neonates contrasts with significant IFN-γ secretion by CMV-infected fetuses and healthy adults. In total, six CMV-infected fetuses were analyzed. The median level of IFN-γ response in the infected population [10% (5–25)] was significantly higher ($p = 0.001$) than in the 10 naive healthy neonates whom we analyzed [0.8% (0.6–1.2)]. Using three-color (CD3/CD8/anti–IFN-γ) cytometry assays, it is possible to determine which CD8$^+$ or CD8$^-$ (*i.e.* CD4$^+$) subset among CD3$^+$ T cells is capable of secreting IFN-γ. As illustrated for three distinct fetuses in Figure 2, a higher proportion of CD8$^+$ T cells than CD8$^-$ T cells (27.5–67.5% *versus* 4.9–7.9%) were capable of secreting IFN-γ in CMV-infected fetuses.

Quantification of cells producing IFN-γ after stimulation with the CMV antigen. The feasibility of detecting and quantifying IFN-γ responses to CMV antigen was demonstrated by comparing responses in peripheral blood from healthy adults exposed to the virus (people from our staff) with responses in a naive population of healthy neonates (cord blood). Among 10 subjects who were exposed to the virus, the mean frequency of T cells producing IFN-γ after stimulation with the CMV antigen was 0.17%, whereas among 10 neonates, the mean CMV antigen–specific effector frequency for this cytokine was at background (−0.03%). Using the same method, IFN-γ responses to CMV by CD8$^+$ T cells could also be detected in adults because the mean frequency of CD8$^+$ T cells producing IFN-γ was 0.11% in the 10 adults *versus* at background (−0.07%) in the 10 neonates. On the basis of these preliminary data, we numerated T cells secreting IFN-γ after stimulation with the specific viral antigen in four fetuses infected with CMV. Consistent IFN-γ response by T cells to the nonspecific Ca^{2+}/PMA activator in these four fetuses (mean, 8%; range, 4.8–11.14%) contrasted with strikingly lower (and even unde-

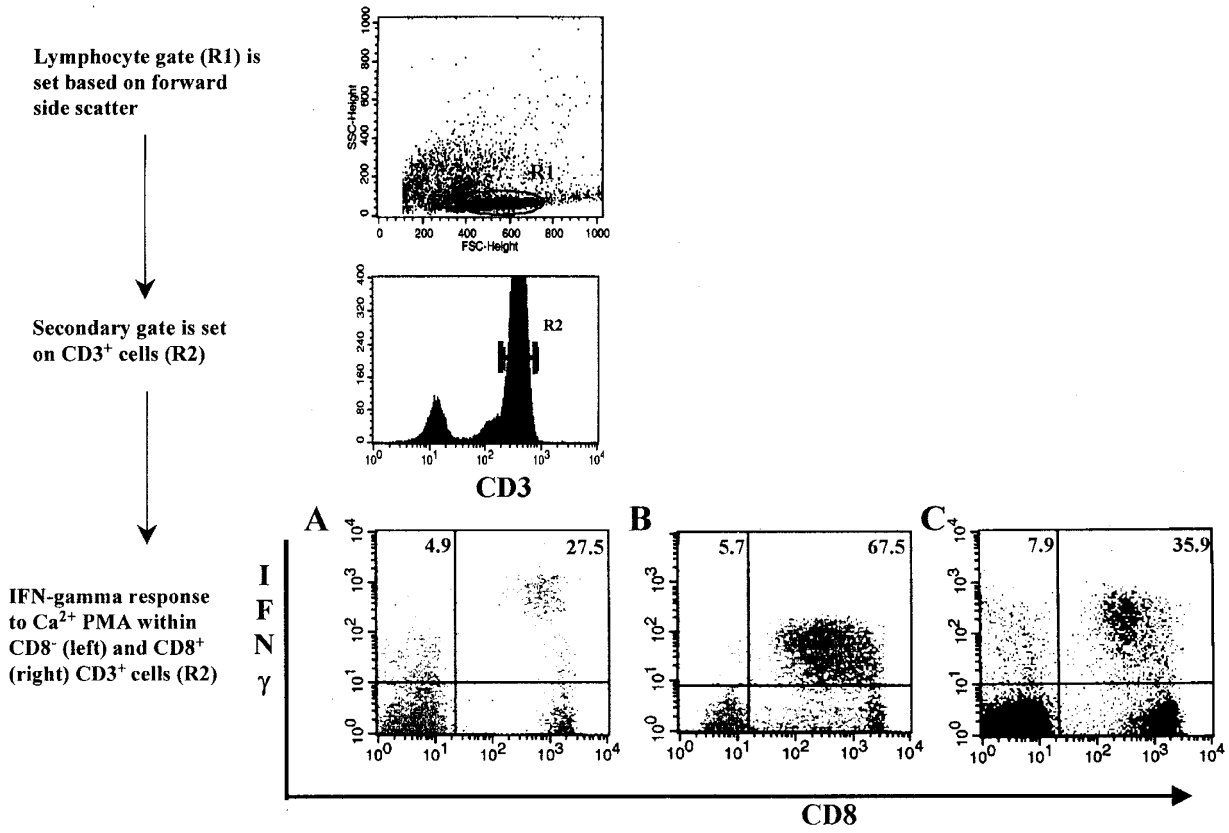

Figure 2. Frequencies of IFN-γ–secreting cells among $CD8^+$ and $CD8^-$ (*i.e.* CD4) T cells after stimulation with Ca^{2+}/PMA. After stimulation as in Figure 1, lymphocyte gate R1 is set based on lymphocyte forward and side scatter characteristics. Then a secondary gate (R2) is set on $CD3^+$ cells. The frequencies of IFN-γ–secreting cells among $CD8^-$ (upper left signals/upper + lower left signals × 100) and among $CD8^+$ cells (upper right signals/upper + lower right signals × 100) are given in the upper quadrants. The results for three independent assays performed in three distinct fetuses infected with CMV are provided.

tectable in some patients) T-cell responses to the CMV antigen (mean, 0.6%; range, −0.01% to 1.84%).

DISCUSSION

We have documented the CD8 T-cell immune response in congenital human CMV infection. In addition to a switch of a proportion of circulating T cells from a naïve ($CD45RA^+$) to a memory ($CD45RA^-$) state, immunophenotyping assays revealed a gain of HLA-DR and a loss of CD28 expression on $CD8^+$ T cells in infected fetuses as soon as the 22nd week of gestational age (the youngest fetus analyzed). Increased expression of HLA-DR (5) and loss of CD28 (personal observation) has been previously found in neonates infected with HIV and also in neonates with congenital *Trypanosoma cruzi* infection (17). Therefore, the activation and differentiation to terminally differentiated phenotypes of $CD8^+$ T cells could occur very early after birth (HIV) or even before birth (*Trypanosoma*). *In vivo* exposure of fetuses to CMV leads to accumulation of activated and terminally differentiated $CD8^+$ T cells as soon as the 22nd week of gestation or even before. These phenotypic changes mimic those previously described in adults during primary infection with viruses. It is interesting that these changes have been associated with the clearance of herpes viridae viruses from the blood and with the development of protective immunity in adults (17).

The induction of CD8 T-cell functional responses to viruses is age-dependent and much weaker in early age than in adults (13–16). Among $CD8^+$ T cells, those that produce IFN-γ are believed to play an important role in the control of CMV replication. Our results show that CMV infection in fetuses increases considerably the frequency of $CD8^+$ T cells producing IFN-γ after stimulation with Ca^{2+}/PMA. This indicates that these cells are endowed with an effector machinery. Significantly fewer cells produced IFN-γ after specific stimulation with the CMV antigen. This difference did not result from inefficient generation of HLA class I or HLA class II peptides from CMV antigen because the antigenic preparation that was used in this study was previously shown to induce IFN-γ responses by both CD4 and CD8 T cells in CMV^+ adults (18). Furthermore, we have demonstrated that CMV-induced cytokine production by $CD3^+$ or $CD8^+$ T cells in our flow cytometric assay correlates with exposure to the CMV antigen. Three nonexclusive mechanisms can be evoked to explain this dissociation. First, in congenital CMV infection, most IFN-γ–secreting cells might not be specific for the CMV antigen. Recently, a new technique using HLA class I peptide tetrameric complexes has been developed for enumerating antigen-specific $CD8^+$ T cells (19). One general observation using this method has been that the massive expansion of $CD8^+$ T cells during acute viral infections is indeed caused by antigen-

specific T cells and cannot be explained by bystander activation (20, 21). Although enumeration of $CD8^+$ T lymphocytes was not feasible in fetuses for technical reasons (small size of blood samples), the possibility that the great majority of memory $CD8^+$ T cells are not virus-specific selectively in congenital CMV infection seems unlikely. A second hypothesis is a stimulation by the specific antigen *in vitro* suboptimal to detect IFN-γ secretion in fetuses. There is no universal stimulation protocol to detect IFN-γ–producing cells. Some studies used extensive co-stimulation (18, 22). We chose to limit T-cell stimulation to presentation of antigen without co-stimulation because subtle defects in T-cell responsiveness may be masked when the stimulation is too strong. Finally, the last hypothesis, which is the one that we favor, is that CMV-specific T lymphocytes are not fully functional in fetuses. In line with this interpretation, an increased number of studies concluded to impaired function of $CD8^+$ T cells based on data showing a dissociation between the number of $CD8^+$ T cells primed with viruses and the number of T cells that respond to the corresponding viral antigens by IFN-γ production (23–26). Comparing IFN-γ responses with CMV during primary infection in both fetuses and adults should definitely demonstrate whether CD8 response to CMV is less intense in early age. Unfortunately, this is complicated given the difficulties of studying clinically silent primary infections in adults.

In addition to $CD8^+$ T cells, $CD4^+$ T cells are required for control of viral infections. They contribute to viral clearance through a variety of mechanisms, including help for $CD8^+$ T cells (27, 28). Although the CD8 T-cell response predominates in CMV-infected fetuses, we also detected, to a lesser extent, CD4 T-cell responses. They include a shift of a proportion of $CD4^+$ T cells to a memory phenotype and also a priming to produce IFN-γ after Ca^{2+}/PMA stimulation. Although present, these responses might be quantitatively insufficient to promote vigorous CD8 T-cell responses. This scenario is suggested by previous reports that have indicated poor T-cell proliferative responses to the CMV antigen in neonates infected with CMV (29, 30). In addition, decreased CD4 T-cell count during congenital infection (this article) might contribute in shortening $CD8^+$ T-cell response.

CONCLUSION

In conclusion, our results provide new data in the delineation of CD8 T-cell responses in congenital CMV infection. They indicate that expansion of CD8 T cells endowed to secrete IFN-γ occurs since 22 wk of gestation in response to CMV infection. These results do not exclude, however, that cytokine production *in vivo* in response to the specific viral antigen can be less vigorous in fetuses than in more mature individuals.

Acknowledgment. The cooperation of all patients and control subjects is gratefully acknowledged. We thank Christophe Merlette, Catherine Martinet, and Valérie Broc for excellent technical assistance; Sandie Samb and Nadine Battery for typing the manuscript; and Corine Alberti for advice regarding statistical analysis.

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