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CASE REPORT

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Changes of $CD8^{+}CD28^{-}$ Tregs and Gamma-Delta-T-cells in a Neonate with Intrauterine Cytomegalovirus Infection: A Case Report

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Abstract

Background Congenital cytomegalovirus (cCMV) infection can lead to a range of adverse outcomes. The majority of cCMV neonates with clinical symptoms are infected postnatally; however, established cases of intrauterine infection are uncommon, resulting in a paucity of reports on clinical findings and lymphocytes expression in CMV-infected neonates.

Case presentation We followed a neonate with cCMV infection from the onset of hospitalization to several months of follow-up. This infant was intrauterine CMV-positive in the amniotic fluid of the mother at 21 weeks' gestation and received intravenous ganciclovir infusion and sequential oral valganciclovir after birth. The typical clinical signs manifested in the nervous system, liver, and peripheral blood and were documented during the hospitalizaion period and up to the follow-up visit. Flow cytometry was employed to examine the expression of T cells, their subsets, and the associated cytokines in peripheral blood samples at various time points. The flow data for the cCMV neonate were compared with those of the controls at each time point. Following treatment, clinical symptoms improved and the infant became CMV negative. However, developmental delays occurred later in life. The proportion of $CD8^{+}CD28^{-}$ Tregs in the peripheral blood of the neonate with congenital CMV infection was higher than that in the controls at the three time points. The expression levels of perforin and granzyme B secreted by γδ T cells (Vδ1 and Vδ2 T cells), increased during the course of hospitalization until follow-up and were higher than those in the controls at the three time points.

Conclusions Despite the alleviation of clinical symptoms, developmental delay in later life remains inevitable in this intrauterine cCMV neonate. $CD8^{+}CD28^{-}$ Tregs and Vδ1 and Vδ2 T cells secreting perforin and granzyme B may be involved in congenital CMV infection, although this hypothesis requires validation in a larger study. This report may contribute to our understanding of the effect of current treatment and the immune status of intrauterine cCMV-infected neonates.

Keywords Congenital CMV infection, Neonate, $CD8^{+}CD28^{-}$ Tregs, Γδ T cells, Vδ1 and Vδ2 T cells

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Background

Congenital cytomegalovirus (cCMV) infection can result in a range of adverse outcomes, including stillbirth, neonatal death, neurological deafness, neurodevelopmental disorders, and liver complications [1]. It is estimated that between 35 and 40% of pregnant women with a primary CMV infection transmit the virus to their fetuses, with approximately 10% of infected fetuses exhibiting symptoms at birth. Furthermore, while some newborns may not display overt ymptoms of CMV infection, they may still develop neural deafness caused by CMV infection later in life [2].

Studies in different regions of China revealed a prevalence of cCMV between 0.23 and 0.7%, with 10 to 15% of cases resulting in symptomatic births [3, 4]. Cases presenting with multiple-organ damage at birth are less common, and cases with severe neurological lesions are even rarer. A review of the cases at our hospital over the past three years reveals that there were 60,000 deliveries, with nearly 10,000 cases discharged from the neonatal department. However, only four cases were diagnosed with multisystem damage due to cCMV and administered antiviral drugs, of which two cases exhibited neurological lesions.

This neonate represents a rare case due to the presence of multi organ dysfunction (liver, haematological system, and lungs) and neurological lesions at birth. Secondly, the neonate was confirmed to have an intrauterine congenital infection with a clear route of intrauterine transmission. This was evidenced by the positive quantification of CMV DNA in the maternal amniotic fluid at mid-pregnancy and in the neonate's blood and urine. However, it was not possible to ascertain with certainty whether the other cases were perinatal or postpartum infections. Furthermore, the neonate was followed from the outset of the hospitalization until the follow-up visit. The clinical findings and laboratory tests at different time points were recorded and analyzed. Additionally, it proved challenging to obtain fresh peripheral blood samples from the neonate at different time points and corresponding controls for flow cytometry. The timeline is presented in Fig. 1A.

Case presentation

Patient and treatment

The infant was delivered spontaneously at home on the evening of 23 April 2022, at a gestational age of 35+6 weeks. Consequently, the amniotic fluid, umbilical cord, placenta, and Apgar scores were unavailable at the time of birth. The infant was observed to cry immediately after birth, as described by the father. The infant was subsequently transferred to the local hospital one hour after birth by an emergency medical service. The infant's weight and length were recorded as 1570 g and 41 cm, respectively. The initial blood test of the neonate at the local hospital demonstrated a diminished platelet count (59*10^9/L), elevated direct bilirubin (33.8 μmol/L) and total bilirubin (89.8 μmol/L), a normal leukocyte count, C-reactive protein (CRP), and alanine aminotransferase (ALT) levels. On day 5, four days later, the platelet count decreased to 44*10^9/L, while the direct bilirubin level increased to 78.7 μmol/L and the total bilirubin reached 129.7 μmol/L. The initial quantification of CMV DNA in urine was 1.41e+7 copies/mL (see Supplementary Table 1). Due to the limited resources available at the local hospital, the infant was transferred to our tertiary neonatal intensive care unit (NICU) on 29 April 2022 (day 6) to receive more specialized care. The neonate was initially diagnosed with congenital CMV infection, preterm low birth weight, small for gestational age, thrombocytopenia, and neonatal cholestasis. In accordance with the established guidelines, ganciclovir (6 mg/kg, q12 h) was administered intravenously from day 7 until discharge, and oral ursodeoxycholic acid (10 mg/kg/day) was initiated on day 9 (Fig. 1A). The patient was treated with intravenous ganciclovir for 3 weeks in our hospital, and then switched to oral valganciclovir for a further 3 weeks (there was no oral valganciclovir in our hospital, and the neonate was taken to the other hospital for valganciclovir and re-evaluated). Following a 6-week course of antiviral treatment, the neonate was CMV negative. The physician at the hosptital providing valganciclovir decided to discontinue valganciclovir (the treatment at the other hospital was described by the family members, more specific reasons unknown).

Diagnosis

The obstetric examination of the mother of the newborn suggested a high risk of trisomy 21 with intrauterine growth retardation. The antenatal ultrasound examination revealed a significantly reduced fetal head circumference in comparison to the gestational age. Additionally, the fetal head exhibited sonographic alterations, and the possibility of intracranial infection could not be excluded. Amniocentesis was performed at 21 weeks of gestation in our hospital. The results of the karyotype and microarray analysis were negative. However, the concentration of CMV nucleic acid in the amniotic fluid was markedly elevated at 2.76e+7 copies/mL. The diagnosis of congenital CMV infection was established, and the route of intrauterine transmission was identified. The infant exhibited typical multi-organ dysfunction (central nervous system, liver, haematological system, and lungs) at birth, with positive quantification of CMV DNA in maternal amniotic fluid of the mother at mid-pregnancy and in neonatal blood and urine. Polymerase chain reaction (PCR) was employed to examine the cerebrospinal fluid, serum, and urine for the presence of CMV nucleic

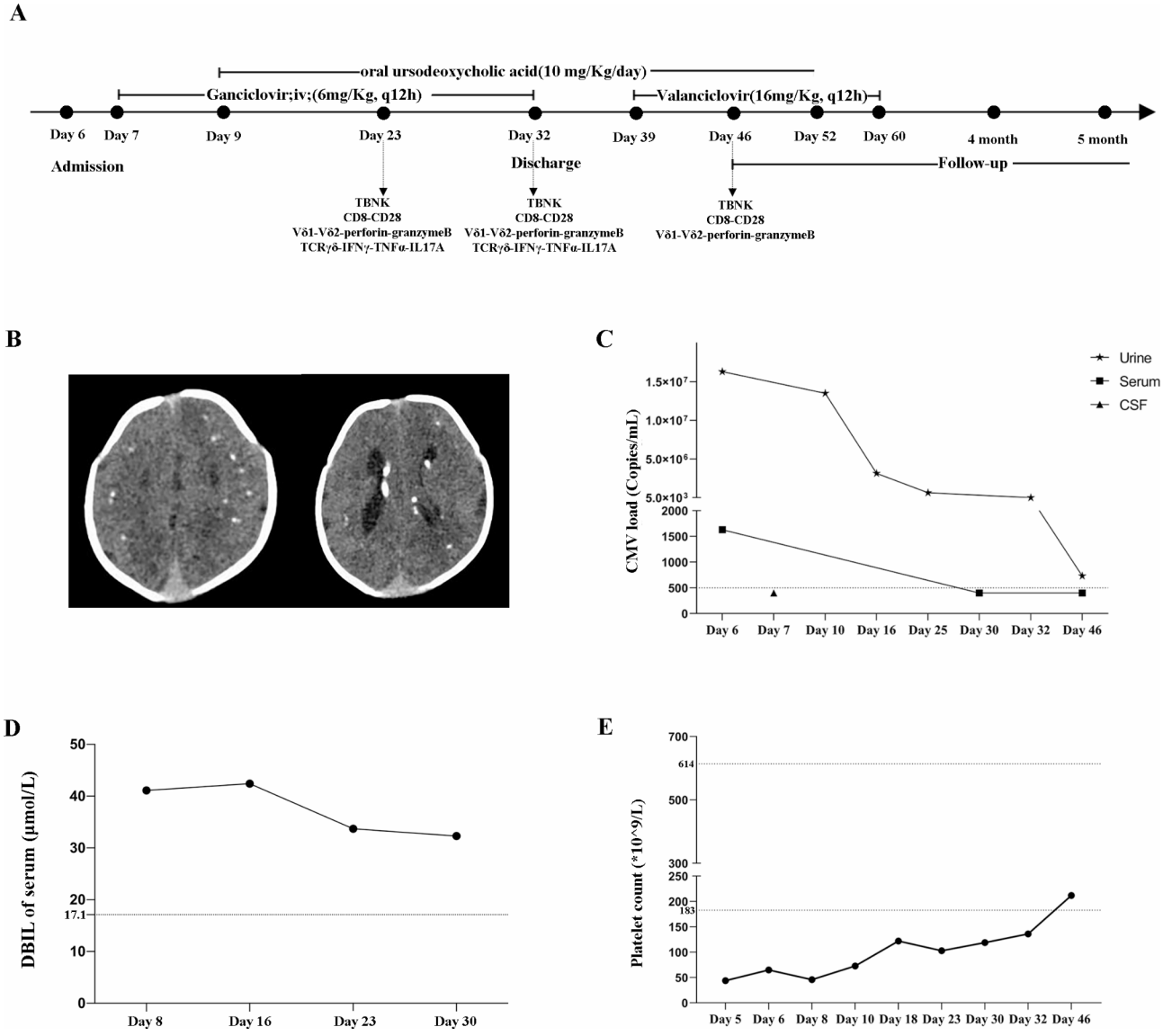

Fig. 1 Timeline of this study and clinical manifestations of the cCMV neonate. (**A**) The long arrow represents the timeline of peripheral blood sampling for flow cytometry and treatments. (**B**) Calcifications in the brain. Calcifications scattered in bilateral cerebral hemispheres (left) and lateral paraventricular (right). White dots indicate calcifications. (**C**) The viral load of CMV was determined in urine, serum, and cerebrospinal fluid (CSF) at various time points. The cut-off is indicated by the dashed line; a value below 500 indicates a negative CMV stauts. (**D**) DBIL (direct bilirubin) was tested in serum at different time points. The dashed line indicates the normal level of DBIL, and the value higher than 17.1 indicates pathological elevation. (**E**) Platelet count in peripheral blood was tested at different time points. The dashed lines incidate the reference range (183–614), and the value lower than 183 indicates thrombocytopenia

acid, in acccordance with the instructions provided by the manufacturer [5] (Human Cytomegalovirus Nucleic Acid Quantification Test kit, DAAN GENE, Guangzhou, China).

Clinical findings

Typical calcifications were observed in both cerebral hemispheres, the lateral ventricles, and the right cerebellar hemisphere, with CT values ranging from 125 to 230 HU. The bilateral ventricles exhibited asymmetry and slight dilation, while the bilateral anterior ventricular angle distance was widened (Fig. 1B). On the third day of admission, the infant exhibited dyspnoea and pulmonary rales, leading to a diagnosis of CMV-associated pneumonia. The infant was treated with nCPAP-assisted ventilation for one day and high-flow oxygen for five days. The levels of CMV DNA in serum and urine were quantified by PCR at various time points, demonstrating a declining trend throughout the course of antiviral therapy. The patient's cerebrospinal fluid was CMV negative (Fig. 1C). The direct bilirubin level exhibited a decline throughout the course of therapy (Fig. 1D). The platelet count

demonstrated an increase from the time of admission to the follow-up assessment on day 46 (Fig. 1E). The trends in key blood counts and significant liver function tests are illustrated in Supplementary Figs. 1 and 2. The brainstem auditory evoked potentials were within the normal range. Following a 26-day hospitalization period, the patient was discharged and continued to receive valganciclovir and ursodeoxycholic acid.

Subsequent to discharge, a series of assessments were conducted at our institution and at the hospital where the oral medications were dispensed. These included evaluations of the patient's blood count, liver function, regular virological nucleic acid monitoring, and neurodevelopmental follow-up. The neonate exhibited the following clinical manifestations: (1) The imaging results revealed multiple cerebellar gyrus, giant gyrus, and abnormal white matter signalling, as well as post-cerebellar injury changes and bilateral ventricular dilatation. These findings were observed through nuclear magnetic resonance imaging (MRI). (2) The infant exhibited mild hearing abnormalities, an abnormal electroencephalogram (EEG), and microcephaly. Following a 20-day course of treatment, the CMV test yielded a negative result, prompting the discontinuation of valganciclovir. At six and seven and a half months of age, the neonate was admitted to the paediatric rehabilitation unit of our hospital for a period of six weeks, during which time he was diagnosed with "brain injury syndrome". Eight months after birth, the neonate was evaluated as an inpatient in the paediatric rehabilitation unit, and the following observations were made: (1) Gross motor: head stable, can raise the head about 90° with elbow support; no delay in pulling up; can roll from supine to lateral position, but can't complete the rollover; when no hand support in prone position, can sit with both hands to support leaning for a few moments and lift the armpit can support part of the body weight; pointy feet. (2) Fine motor: both hands clench fists, have hand-mouth movement, can hold hands together, can hold flower bell stick, cannot grasp objects; (3) Speech: can pronounce single rhyming sounds; can laugh, but is not very responsive to sound; does not vocalise to people or objects; (4) Social adaptation: can smile, look at people's faces, respond to teasing, cannot turn head to look for sound source; (5) Others: increased muscle tone in all four limbs, ATNR (±), standing drape (+).

Flow cytometry analysis

Peripheral blood samples were obtained from the cCMV neonate on day 23 (during treatment), day 30–32 (discharge), and day 46 (follow-up) for laboratory analysis. Flow cytometry was employed to ascertain the proportion of circulating T cells ($CD4^+$ and $CD8^+$ T cells), B cells, natural killer (NK) cells, $CD8^+CD28^-$ T regulatory cells (Tregs), and γδ T cells (Vδ1 and Vδ2 T cells). The objective was to determine the expression levels of tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-17, perforin, and granzyme B secreted by γδ T cells in the peripheral blood of the cCMV neonate at the three time points. At each time point, the flow data of the cCMV neonate were compared with those of the controls. The demographic data of the case and controls are presented in Supplementary Table 2. The peripheral blood of the healthy neonates of the same age as the CMV-infected neonate served as controls. A total of 29 healthy neonates from Shenzhen Baoan Women's and Children's Hospital were included in this study with the consent of their guardians.

For surface marker staining, freshly isolated PBMCs (1×10^6/mL) were washed and incubated with monoclonal antibodies. For TBNK staining, a commercially available kit (Mandary, China) was utilised, containing two panels (T cell panel: Percp-anti-CD45, FITC-anti-CD3, APC-anti-CD4, PE-anti-CD8; B-NK panel: Percp-anti-CD45, FITC-anti-CD3, APC-anti-CD19, and PE-anti-$CD16^+CD56^-$); for staining of $CD8^+CD28^-$ Tregs, the antibodies used were FITC-anti-CD8 and APC-anti-CD28.

For perforin and granzyme B staining, isolated PBMCs were washed and stained with Alexa Fluor 700-anti-CD3, Brilliant Violet 421-anti-TCRγδ, PE/Cy7-anti-Vδ2 (all from BioLegend, San Jose, CA, USA), and FITC-anti-Vδ1 (Genetex, Germany). Subsequently, the cells were fixed, permeabilized, and stained with Alexa Fluor-647-anti-perforin and Brilliant Violet 510-anti-granzyme B (BD Bioscience) in accordance with the manufacturers' insturctions.

Prior to staining for intracellular cytokines secreted by γδ T cells, isolated PBMCs were suspended in RPMI 1640 medium supplemented with 10% FBS and stimulated with phorbol-12-myristate 13-acetate (PMA) (50 ng/mL), ionomycin (1 μg/mL), and brefeldin (1 μg/mL) (all from Biogems, Rocky Hill, NJ, USA) for 4 h in 5% CO_2 atmosphere at 37 ℃. Subsequently, the cells were washed and stained with Alexa Fluor 700-anti-CD3 and Brilliant Violet 421-anti-TCRγδ in the dark at room temperature for 20 min. Following these staining, the cells were fixed, permeabilized, and stained with Alexa Fluor 647-anti-IL17A, PE-anti-TNFα, Brilliant Violet 605-anti-INFγ (BioLegend) according to the manufacturers' protocol. The panels and antibodies utilized in the study are detailed in Supplementary Tables 3 and 4.

The labeled cells were quantified using the BD Fortessa system (BD Bioscience) and the Bricyte E6 (Mandary, China), and the data were analyzed using Flowjo software (Tree Star). The gating strategy of different lymphocytes is illustrated in Supplementary Figs. 3–6.

Table 1 Comparison of TBNK lymphocytes between the cCMV neonate and controls

Lymphocytes parameters	Day24		Day30-32	
	cCMV neonate	Controls	cCMV neonate	Controls
		M(P25 ~ P75)		M(P25 ~ P75)
Lymphocytes numbers (/μl)	2635	2266 (1932 ~ 2630)	1645	1960 (1645 ~ 2255)
Total T cell numbers (/μl)	1498	1642 (1547 ~ 2108)	748	1487 (1257 ~ 1765)
Total T (%)	56.86	79 (75 ~ 81)	60.23	79 (73 ~ 82)
$CD4^+$T cells numbers (/μl)	1025	1211 (1098 ~ 1247)	598	1029 (923 ~ 1219)
$CD4^+$T cell (%)	38.90	53 (50 ~ 58)	40.87	56 (50 ~ 61)
$CD8^+$T cells numbers (/μl)	462	426 (354 ~ 882)	218	447 (310 ~ 492)
$CD8^+$T cell (%)	17.52	20 (17 ~ 25)	18.0	20 (16 ~ 24.5)
CD4/CD8	2.22	2.61 (2.11 ~ 3.34)	2.34	2.91 (2.16 ~ 3.47)
B cell numbers (/μl)	745	287 (230 ~ 367)	350	365 (170 ~ 464)
B cell (%)	28.88	12 (11 ~ 15)	20.85	11 (9 ~ 15)
NK cell numbers (/μl)	294	131 (90 ~ 240)	264	97 (64 ~ 192)
NK cell (%)	11.4	8 (5 ~ 13)	17.20	3 (3 ~ 5)

Data of the cCMV neonate was presented. Data of healthy controls was presented as median (the figure outside the brackets) and quartile with percent 25 ~ percent 75 (the figures in the brackets)

Flow cytometry results

The proportion of T cells in the lymphocytes of the cCMV neonate was lower than that of the controls at both day 24 (58.86% vs. 79%, interquartile range (IQR)=75–81) and day 30–32 (60.23% vs. 79%, IQR=73–82) (Table 1). Additionally, the proportions and numbers of other lymphocytes are presented in Table 1.

The subsets of T cells are then examined. $CD8^+CD28^-$ Tregs play a suppressive regulatory role in several disease states [6, 7]. The proportion of $CD8^+CD28^-$ Tregs among the $CD8^+$ T cells demonstrated a declining trend from day 24 to day 30–32, followed by an increase until day 46. In comparison to the corresponding controls, the proportion of $CD8^+CD28^-$ Tregs was higher in the cCMV neonate at day 24, day 30–32, and day 46, respectively (Table 2; Fig. 2A).

Given the crucial role of γδ T cells in the immune response to CMV infection [8], we further investigated the production of TNF-α, IFN-γ, and IL-17 A by γδ T cells in the cCMV neonate. The expression levels of TNF-α, IFN-γ, and IL-17 A were higher on day 24 than on day 30–32. The expression levels of TNF-α and IFN-γ at both time points in the cCMV neonate were higher than those in the corresponding controls, whereas the expression levels of IL-17 were lower than those in the controls on day 30–32 (Table 2).

Vδ1 and Vδ2 T cells represent two major subsets of γδ T cells that play pivotal roles in CMV infection [8]. The proportion of Vδ1 T cells in CD3 exhibited a decline from day 24 to day 30–32, followed by an increase until discharge. In contrast, the proportion of Vδ2 T cells demonstrated a similar pattern from day 24 to day 30–32, before undergoing a decline from day 30–32 to day 46. (Table 2). It is noteworthy that both perforin and granzyme B, secreted by Vδ1 and Vδ2 T cells, demonstrated an increasing trend from the onset of hospitalization to discharge. Moreover, the expression levels of perforin and granzyme B secreted by Vδ1 and Vδ2 T cells were higher than those of the controls at all three time points (Table 2; Fig. 2B, C, D, E).

Discussion and conclusions

We followed a cCMV neonate with typical symptoms and a clear route of intrauterine transmission from the beginning of hospitalization to the follow-up visit, which is a rare occurrence. The prevalence of cCMV in China has been estimated to be between 0.23% and 0.7% [3, 4], while in developed countries, it is thought to be between 0.14% and 0.7% [9]. However, the majority of cCMV

Table 2 The fraction of T cell subsets of the cCMV neonate and controls at day 24, day 30–32, and day 46

T cell subsets	Day 24		Day 30–32		Day 46	
	cCMV neonate	Controls	cCMV neonate	Controls	cCMV neonate	Controls
		M(P25 ~ P75)		M(P25 ~ P75)		M(P25 ~ P75)
$CD8^+CD28^-$(%)	15.9	4.05 [2.69, 5.48]	12.54	2.33 [1.71, 4.35]	15.2	2.42 [1.80, 4.34]
$TCR\gamma\delta^+TNF\alpha^+$(%)	82.3	45.30 [39.95, 46.85]	50.1	28.90 [17.80, 30.20]	NA	NA
$TCR\gamma\delta^+IFN\gamma^+$(%)	68.4	11.50 [9.77, 19.20]	30.6	6.43 [3.14, 10.66]	NA	NA
$TCR\gamma\delta^+IL17A^+$(%)	0.84	0.44 [0.40, 0.70]	0.38	0.82 [0.53, 1.05]	NA	NA
$CD3^+V\delta1^+$(%)	1.0	0.75 [0.46, 0.97]	0.55	0.50 [0.41, 0.80]	0.95	0.60 [0.24, 0.84]
$CD3^+V\delta1^+perforin^+$(%)	2.28	0.40 [0.18, 0.78]	12.8	2.05 [1.40, 3.23]	13.9	2.69 [1.40, 3.55]
$CD3^+V\delta1^+granzymeB^+$(%)	3.37	1.08 [0.58, 1.52]	14.1	2.10 [1.40, 3.11]	18.1	2.64 [1.48, 3.26]
$CD3^+V\delta2^+$(%)	2.34	1.16 [0.73, 1.95]	2.39	1.24 [0.70, 3.00]	1.49	3.33 [2.03, 3.88]
$CD3^+V\delta2^+perforin^+$(%)	25.5	1.10 [0.67, 1.63]	52.3	1.78 [0.60, 2.68]	70.9	5.19 [1.78, 11.36]
$CD3^+V\delta2^+granzymeB^+$(%)	24.8	5.55 [3.88, 6.96]	55.5	9.80 [5.85, 14.70]	78.3	21.60 [6.90, 35.90]

NA : not available

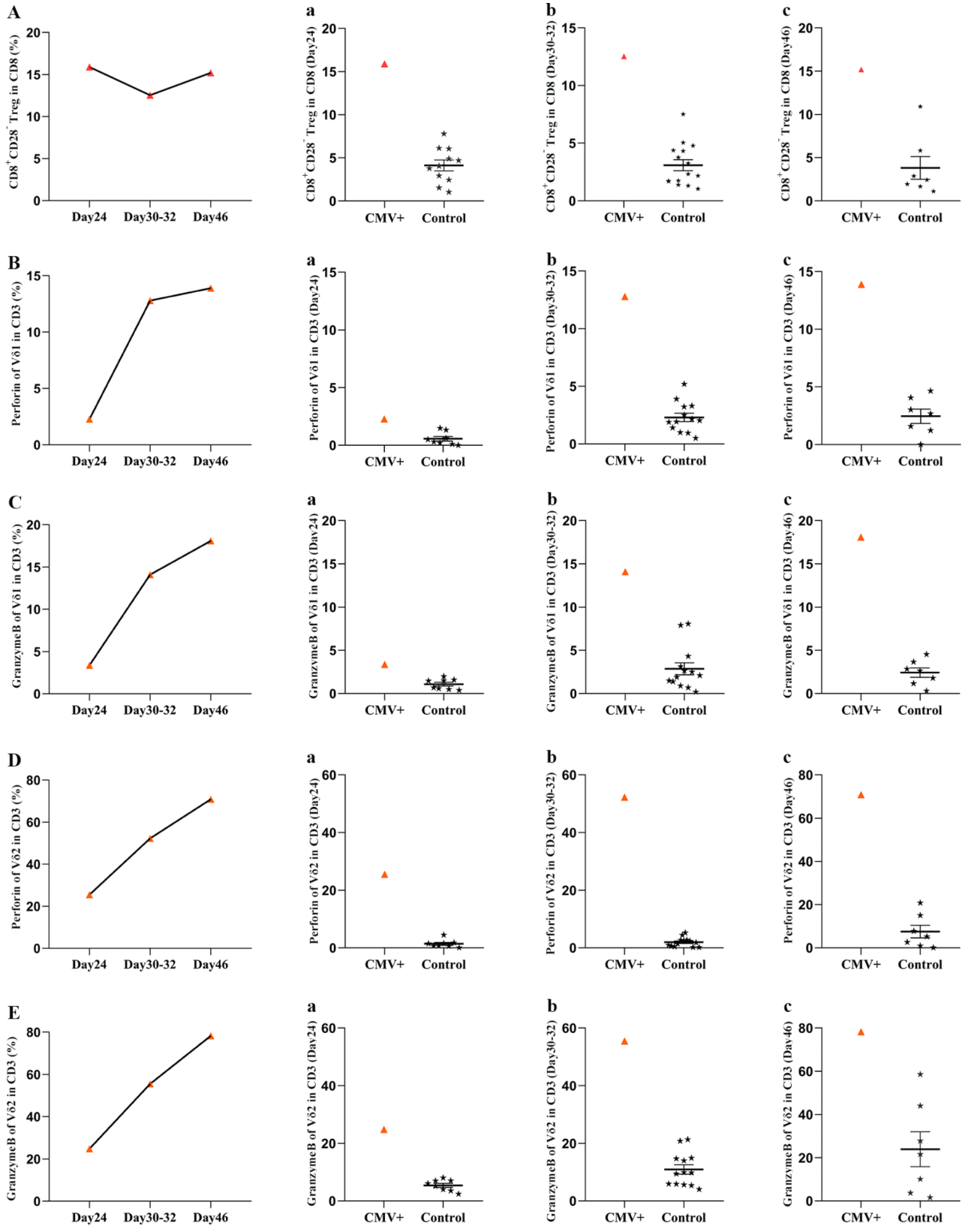

Fig. 2 (See legend on next page.)

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Fig. 2 The proportion of $CD8^+CD28^-$ Tregs and the expression of Vδ1 and Vδ2 T cells secreting perforin and granzymeB in the cCMV neonate. (**A**) The changes in the proportion of $CD8^+CD28^-$ Tregs out of $CD8^+$ T cells during the antiviral treatment of the cCMV neonate. The comparison of proportion of $CD8^+CD28^-$ Tregs out of $CD8^+$ T cells between the cCMV neonate and the controls at day 24 (**a**), day 30–32 (**b**), and day 46 (**c**). The changes in Vδ1 T cells secreting (**B**) perforin and (**C**) granzyme B during antiviral therapy of the cCMV neonate. A comparison of Vδ1 T cells secreting perforin and granzyme B between the cCMV neonate and controls at day 24 (**a**), day 30–32 (**b**), and day 46 (**c**). The changes in Vδ2 T cells secreting (**D**) perforin and (**E**) granzyme B during the antiviral therapy of the cCMV neonate. A comparison of Vδ2 T cells secreting perforin and granzyme B between the cCMV neonate and controls at day 24 (**a**), day 30–32 (**b**), and day 46 (**c**)

neonates are born asymptomatic. Furthermore, there are even fewer cases of multiple organ dysfunction at birth, and even fewer cases of severe neurological lesions. A search for cases of CMV diagnosed in our hospital in the last 3 years revealed that the total number of deliveries in our hospital was 60,000, with nearly 10,000 cases were discharged from the neonatal department. Nevertheless, only four cases were diagnosed with multi-system damage due to cCMV and treated with antiviral drugs, two of which exhibited neurological lesions. Our case presented with the typical multi-organ dysfunction and neurological lesions observed in such instances. It is noteworthy that only our case was confirmed as an intrauterine infection, whereas the other cases were perinatal or postpartum infections. In addition, the acquisition of fresh peripheral blood samples from the neonate at varying time points, along with corresponding controls for flow cytometry, is an uncommon occurrence.

The patient was administered intravenous ganciclovir for a period exceeding three weeks, followed by oral valganciclovir to maintain antiviral therapy. The clinical presentation, blood test, and CMV DNA quantification demonstrated favourable treatment outcomes and the presence of leukopenia, which did not necessitate intervention [10]. Despite the absence of CMV DNA in the cerebrospinal fluid, the presence of typical foci of calcification in the brain provides evidence of CMV infection of the central nervous system (CNS) during early or mid-pregnancy. The negative result may be atrributed to the activation of the immune system, which effectively eliminates the virus following intrauterine neurological CMV infection. However, the presence of multiple abnormal neurological manifestations and poor prognosis (as evidenced by abnormal EEG, brainstem evoked potentials, GM and cranial imaging during hospitalization and follow-up, as well as significant microcephaly and developmental delay) indicates a high incidence of long-term sequelae following CMV CNS infection. Consequently, regular follow-up is essential to assess hearing and mental development.

$CD8^+CD28^-$ Tregs have been associated with the presence of CMV infection [11]. Compared to the corresponding controls, the proportion of $CD8^+CD28^-$ Tregs was higher in the cCMV neonate at the three time points, which is in consistent with the findings of other reports. In patients with rheumatoid arthritis, the proportion of $CD8^+CD28^-$ Tregs is higher in those with CMV-positive rheumatoid arthritis than in those with CMV-negative rheumatoid arthritis [12]. Additionally, CMV infection induced an elevation in $CD8^+CD28^-$ Tregs in immunosenescent patients [13]. These findings suggest that $CD8^+CD28^-$ Tregs are not only implicated in diverse disease states but also exhibit an increasing prevalence during CMV infection.

γδ T cells secrete substantial quantities of TNF-α and IFN-γ when cultured with CMV-infected cells, suggesting a potential correaltion between inflammation and CMV infection [14, 15]. Additionally, our findings revealed elevated levels of TNF-α and IFN-γ secretion by γδ T cells in the cCMV neonate. Vδ1 and Vδ2 T cells represent the predominant subsets of γδ T cells [16]. In the peripheral blood of the cCMV neonate, the proportion of Vδ1 and Vδ2 T cells fluctuated at different time points. However, the number of Vδ1 and Vδ2 T cells that secreted perforin and granzyme B increased. These data suggest that perforin and granzyme B produced by Vδ1 and Vδ2 T cells may be involved in the control of cCMV infection.

In a study of 182 cCMV neonates, Soriano-Ramos et al. employed flow cytometry to assess the role of T cell immune responses and their potential association with the development of long-term sequelae [9]. They found that the number of $CD4^+$ T cells at birth was significantly lower in infants who subsequently developed sequelae compared to those who did not. In our study, the $CD4^+$ T cells count of the neonate with long-term sequelae was lower than that of the healthy controls at two time points. It is important to note that the control groups and clinical status of the neonates in the two studies are not directly comparable. However, both studies demonstrate the significance and potential of the T cell response in cCMV neonates.

It is challenging to ascertain the impact of antiviral therapy or the congenital infection itself on the flow cytometry results, as evidenced by our findings. The neonate was administered antiviral therapy upon her admission to our hospital. It is possible that the higher expression of $CD8^+CD28^-$ Tregs in cCMV may inhibit the killing function of other effector cells, thereby contribute to the latency of the virus. The expression of perforin and granzyme B continued to increase after treatment, which may be a potential reason for the reduction in CMV and improvement in clinical symptoms.

Nevertheless, further investigation is required to elucidate the underlying mechanisms.

Furthermore, the analysis is constrained by the fact that only a single case of intrauterine cCMV was available for examination. This is due to the rarity of specific cCMV in clinical practice. Despite the fact that our hospital is a maternity hospital with the largest number of deliveries in Shenzhen city and a level 3 neonatal intensive care unit, we have only encountered one case of cCMV with a clear intrauterine infection route in recent years. Obtaining blood samples at three time points for our case was challenging, resulting in the availability of flow data for only two time points for certain lymphocytes. Determining the changes in $CD8^+CD28^-$ Tregs and γδ T cells by flow cytometry is a potential tool for physicians to monitor the immune status of patients. However, further investigation is required to ascertain the suitability of this method for cases diagnosed with cCMV infection. Plans exist to increase the sample size and to collect more clinical neonatal CMV cases, including both congenital and non-congenital CMV, for testing and analysis. This will facilitate future investigations to ascertain whether comparable findings are present.

In conclusion, we have investigated the changes in clinical manifestations and T-cell subsets at different time points in a typical intrauterine cCMV-infected neonate. It was observed that while CMV can become negative after treatment, developmental delay can occur in the infant in later life. The involvement of $CD8^+CD28^-$ Tregs and Vδ1 and Vδ2 T cells secreting perforin and granzyme B in congenital CMV infection is a hypothesis that merits further investigation in a larger study.

List of abbreviations

cCMV	Congenital cytomegalovirus
CMV	Cytomegalovirus
Tregs	T regulatory cells
TNF	Tumor necrosis factor
IFN	Interferon
IL	Interleukin
CRP	C-reactive protein
ALT	Alanine aminotransferase
IQR	Interquartile range
CNS	Central nervous system

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-05051-z.

Supplementary Material 1

Author contributions

XC and ZJ contributed equally to this work. XC and ZJ performed the experiments, analyzed data; PZ collected the clinical data; FJ and TX collected the laboratory data; QT designed and supervised the study, and wrote the manuscript. All authors reviewed the manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

We confirm that all experimental protocols were approved by the ethics committee of Shenzhen Baoan Women's and Children's Hospital and the committee's reference number is LLSC-2022-02-07-29-KS.
We confirm that all methods were performed in accordance with the ethical standards as laid down in the Declaration of Helsinki and its later amendments or comparable ethical standards.
We confirm that written informed consent was obtained from the guardians of all participants.

Consent for publication

We confirm that the consent for publication was obtained from the guardians of all participants.
We confirm that the "written informed consent" was given from the guardians of all participants/patients for their personal or clinical details along with any identifying images to be published in this study.

Competing interests

The authors declare no competing interests.

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