



Cytokine profiles in an extremely preterm infant with congenital syphilis

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Abstract

We report the cytokine profiles of an infant with congenital syphilis as a first case. This female infant was born by vaginal delivery at a gestational age of 27 weeks during her mother's treatment for syphilis. Elevation of T helper (Th)-1 cytokines (interleukin (IL)-2, IL-12) and IL-17, which supports immunological mechanisms of Th-1, was similar to that in cases of syphilis in adults. IL-6 and granulocyte colony-stimulating factor (G-CSF), the synergistic effects of which cause the leukemoid reaction, were also elevated. The levels of interferon- γ and IL-17 in cerebral spinal fluid, which are elevated in neurosyphilis in adults, were slightly elevated.

Key words : congenital syphilis, cytokine, IL-2, IL-12, IL-17

Introduction

Intrauterine infections, including congenital syphilis, may cause chorioamnionitis and are a major cause of preterm labor. Cytokine storm occurs when inflammation spreads to the fetus. This clinical condition is called fetal inflammatory response syndrome (FIRS)¹⁾. In recent years, the number of patients infected by syphilis among young people has been increasing in Japan²⁾. However, the immunological mechanisms for bloodstream infections by congenital syphilis are still unknown³⁾. Furthermore, there have been no reports on cytokine profiles in neonates with congenital syphilis.

Case presentation

The mother was 17 years old and had a natural pregnancy. She began to get prenatal checkups at 23 weeks' gestation, at which time it was revealed that she was infected with syphilis. Oral administration of ampicillin was started at 25 weeks' gestation to treat the syphilis. Ten days after the start of medication, the mother was emergently transported

to our hospital because she went into labor due to an elevated inflammatory response caused by the intra-uterine infection. On the same day, a female infant weighing 912 g was born by vaginal delivery at 27 weeks and 2 days gestation. She was diagnosed as having congenital syphilis with leukemoid reaction by blood examination at 2 hours of age. Laboratory testing revealed a white blood cell (WBC) count of 27,300/ μ L with blasts, C-reactive protein (CRP) 0.85 mg/dL, immunoglobulin M (IgM) 250.7 mg/dL, syphilis rapid plasma reagin (RPR) 283.2 RPR units (RU) (normal value 0 ; in maternal syphilis, RPR 41.2 RU), and fluorescent treponemal antibody-immunoglobulin M (FTA-ABS IgM) 2+ in cerebrospinal fluid (CSF), which suggested neurosyphilis (Table 1). The weight of placenta was 390 g. Histopathological diagnosis of the placenta revealed chorioamnionitis and funisitis. Benzylpenicillin potassium at 100,000 U/kg/day from day 0 to day 7 after birth and 150,000 U/kg/day from day 8 to day 10 after birth was administered intravenously. The WBC count (39,300/ μ L) peaked at 12 hours after birth and then decreased. Blasts were present only at birth and disappeared from peripheral blood (PB). Also, CRP

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Table 1.

Complete blood count		biochemical test			
WBC	27,300/ μ L	TP	4.6 g/dL	TPLA COI	83 COI
blast		Alb	2.2 g/dL	RPR	283.2 RU
stab	3%	LDH	466 U/L		
seg	53%	AST	48 U/L	Cerebrospinal fluid	
Lympho	37%	ALT	13 U/L	TP	170.8 mg/d.L
Hb	14.6 g/dL	D-Bill	2.3 mg/dL	Glu	34 mg/d.L
Plt	214,000/ μ L	T-Bill	0.8 mg/dL	cell count	22/ μ L
		BUN	7.7 mg/dL	mononuclear cells	20/ μ L
Arterial Blood Gas Analysis		Cre	0.43 mg/dL	polynuclear cells	2/ μ L
pH	7.322	CK	51 U/L	FTA IgM	2+
PCO2	39.2	CRP	0.85 mg/dL		
HCO3	19.7	IgG	567.8 mg/dL		
BE	5.4	IgM	250.7 mg/dL		

WBC : white blood cell, Hb : hemoglobin, Plt : platelets, BE : base excess, TP : total protein, Alb : albumin, LDH : lactate dehydrogenase, AST : aspartate aminotransferase, ALT : alanine aminotransferase, D-Bill : direct bilirubin, T-Bill : total bilirubin, BUN : blood urea nitrogen, Cre : creatinine, CK : creatine kinase, CRP : C-reactive protein, IgG : immunoglobulin G, IgM : immunoglobulin M, TPLA COI : treponema pallidum latex agglutination cut off index, RPR : syphilis rapid plasma regain, Glu : glucose, FTA-IgM : fluorescent treponemal antibody-immunoglobulin M

peaked at 1.29 mg/dL at 12 hours of age and then gradually decreased. She was discharged home at age 83 days with no apparent complications due to congenital syphilis.

Methods

We measured serum cytokine levels with the Bio-Plex protein array system (Bio-Rad, Alameda, CA, USA), as described previously, using the Bio-Plex Pro Human Cytokine 17-plex Assay⁴. Cytokine values in cord blood (CB) and PB of +2.0 SD or higher than the average of a control group previously described by us were considered to be abnormally high⁵. For comparison, a case of group congenital listeria ventriculitis⁶, and congenital listeria pneumonia⁷ are included. These cases measured the same cytokines with the same assays as in the present case. Reference intervals for cytokines in CSF are not established for extremely preterm infants.

Ethics

We obtained informed consent from the patient's parents to report this case.

Results

Cytokine profiles of CB and PB at 2 hours after birth revealed that the values of the T helper (Th)-1 cytokines (interleukin (IL)-2, IL-12, interferon

(INF)- γ), Th-2 cytokines (IL-5, IL-10, IL-13), IL-17, IL-6, and granulocyte colony-stimulating factor (G-CSF) were very high compared with control values. Among the proinflammatory cytokines, the values of IL-1 β and tumor necrosis factor (TNF)- α in CB were as high as those in listeria pneumonia. The value of IL-6 was lower than those of congenital listeria ventriculitis and congenital listeria pneumonia. Among the Th-1 cytokines in CB, the elevations of IL-2 and IL-12 were notably higher than those of congenital listeria ventriculitis and congenital listeria pneumonia. Among the Th-2 cytokines in CB, IL-4 was not elevated at all, but the elevations of IL-5 and IL-13 were notably higher than those of congenital listeria ventriculitis and congenital listeria pneumonia (Table 2). The method used to measure cytokine profiles in adult syphilis cases differed from that used in our cytokine profiles. Therefore, we did not present these values in Table 2. Pro-inflammatory cytokines, INF- γ , and IL-17 in CSF were lower than that in the cases of congenital listeria ventriculitis, but the values of INF- γ and IL-17 were slightly elevated (Table 3).

Discussion

To the best of our knowledge, this is the first report of a cytokine profile in an infant with congenital syphilis in the early neonatal period. In this case, syphilis infection in the second trimester of pregnancy caused FIRS while the mother was being

Table 2.

	this case			Lm encephalitis ⁶⁾	Lm pneumonia ⁷⁾	Control ⁵⁾	
	CB(d0)	PB (2h after birth)	PB (2m after birth)	CB(d0)	CB	CB	PB
proinflammatory cytokine							
IL-1b	69.16	267.57	OR	12.74	129.1	1.28±8.93	2.1±1.9
IL-6	49.83	55.84	OR	690.71	5,798.6	4.43±14.3	4.3±3.1
TNF- α	541.07	1,229.84	33.36	19.83	548.3	1.80±3.13	6.6±5.8
Th-1 cytokines							
INF- γ	38.06	71.42	OR	75.46	961.3	1.80±3.13	5.3±5.2
IL-2	49.49	287.53	OR	0	3.1	1.01±9.74	0.54±1.20
IL-12	241.83	983.26	OR	6.3	41.9	0.60±4.23	2.6±4.1
Th-2 cytokines							
IL-4	0	0	OR	1.34	6	0.36±1.54	0.14±0.20
IL-5	150.07	235.57	OR	0.95	5.4	0.76±1.52	2.0±0.5
IL-10	29.84	92.87	1.55	17.27	29.3	0.89±2.03	1.8±2.1
IL-13	57.84	109.73	OR	8.52		3.77±21.2	1.2±1.1
Th-17 cytokine							
IL-17	10.88	51.17	OR	21.6	86.3	1.23±4.83	7.0±7.4
growth factors							
G-CSF	207.14	1,058.01	OR	302.6	2,680.1	9.12±37.0	11.2±7.0
GM-CSF	26.93	39.92	OR	112.02	254.8	4.27±28.7	17.0±13.7
IL-7	29.84	85.07	OR	70.34	2.5	2.31±2.31	0.22±0.33
chemokines							
MCP-1	100.22	240.15	118.2	379.01	1,537.8	82.1±88.1	487.0±2,214
MIP-1b	282.02	455.16	92.58	163.99	252.4	224.2±131.7	188.5±73.1
IL-8	115.05	202.89	29.92	199.4	1,189.9	18.8±33.0	20.0±10.4

CB : cord blood, PB : peripheral blood, OR : out of range below, IL : interleukin, TNF : tumor necrosis factor, INF : interferon, G-CSF : granulocyte colony-stimulating factor, GM-CSF : granulocyte/macrophage colony stimulating factor, MCP-1 : monocyte chemoattractant protein-1, MIP-1 β : macrophage inflammatory protein-1, Lm : listeria
We include cytokine profiles from other coauthored manuscripts (references 6 and 7).

treated for syphilis with ampicillin. Inflammation of FIRS was suspected to have induced the extremely premature birth. We discovered three important facts from the results of this study.

First, and most importantly, elevations of Th-1 cytokines (IL-2, IL-12) and IL-17, which supports immunological mechanisms of Th-1, were similar to those in cases of syphilis in adults. IL-12 is involved in the differentiation of naive T cells into Th-1 cells. The levels of IL-12 were higher than those in listeria. Cell-mediated immunity of congenital syphilis infection is more active than that of listeriosis. IL-2 is essential for the activating function of CD8(+) killer T-cells, which play an important role in syphilis infection. Thus, IL-2 and IL-12 are especially important cytokines in syphilis infection⁸⁾. However, IL-4 was not elevated at all, which is similar to that in cases of syphilis in adults⁹⁾. Furthermore, increased IL-17 has been noted in syphilis infection in adults¹⁰⁾, and the levels

of IL-17 were increased in the present case as well. Compared to those in cases of listeria infection and syphilis in adults, the Th-2 system cytokines were noticeably increased in the present case. However, the value of IL-4, which promotes differentiation into Th-2 cells, was low, and it is unclear why the levels of IL-5 and IL-13 were elevated. Further studies of similar cases are needed.

Second, there are few reports of congenital syphilis complicated by leukemoid reaction in the early neonatal period. We reported that the leukemoid reaction was due to a synergistic effect of IL-6 and G-CSF¹¹⁾. These two cytokines were also elevated in the present infant. The elevation of G-CSF in CB at 2 hours after birth was particularly high. Although elevations of IL-6 and G-CSF caused the leukemoid reaction, the value of IL-6 was itself lower than that in the other infections. It is possible that the level of IL-6 was not remarkably high due to some unknown mechanism. A high

Table 3.

	this case CSF(d0)	Lm encephalitis ⁽⁶⁾ CSF(d11)
proinflammatory cytokine		
IL-1b	1.25	29.94
IL-6	0	51.71
TNF- α	6.03	27.32
Th-1 cytokines		
INF- γ	3.82	62.06
IL-2	1.68	0
IL-12	0	4.82
Th-2 cytokines		
IL-4	0	1.47
IL-5	0	1.37
IL-10	5.9	37.79
IL-13	0	171.08
Th-17 cytokine		
IL-17	0.81	30.88
growth factors		
G-CSF	25.88	552.01
GM-CSF	0	63.95
IL-7	9.83	19.15
chemokines		
MCP-1	564.15	1,694.13
MIP-1b	39.74	155.28
IL-8	299.86	2,198.15

CSF : cerebrospinal fluid, IL : interleukin, TNF : tumor necrosis factor, INF : interferon, G-CSF : granulocyte colony-stimulating factor, GM-CSF : granulocyte/macrophage colony stimulating factor, MCP-1 : monocyte chemoattractant protein-1, MIP-1 β : macrophage inflammatory protein-1 β , GBS : Group B streptococcus, Lm : listeria, We include cytokine profiles from another coauthored manuscript (reference 6).

level of IL-6 is accompanied by the diminished ability of Th-1 lymphocytes¹⁰. It is assumed that the immunological mechanisms of Th-1 are important for congenital syphilis, whereas those of Th-2 are not.

Third, it was reported that in CSF, IL-17 and INF- γ are elevated in neurosyphilis in adults¹¹. In the present case, the cell count in the spinal fluid was not obviously increased. In her CSF, FTA-ABS IgM was clearly positive, and the levels of INF- γ and IL-17 were slightly elevated. Therefore, we diagnosed our patient as having complicated neurosyphilis. The reason why the levels of INF- γ and IL-17 in the infant's CSF were not so high may be because of the medication taken by the mother.

Some cytokines such as IL-2, IL-4, IL-5, IL-8, IL-10, MCP-1, MIP-1, and TNF- α are elevated dur-

ing preterm labor itself¹². However, IL-4 and especially MIP-1b, which is strongly associated with preterm labor, were not elevated in the present case, indicating that these cytokines might be elevated by factors other than preterm labor.

Conclusion

We investigated cytokine profiles in an extremely premature infant with congenital syphilis. As is characteristic in adult cases, the Th-1 cytokines and IL-17 were increased in this infant. It will be necessary to accumulate similar cases to elucidate a more definitive pathogenesis of such congenital syphilis infection.

Disclosure

The authors declare no conflicts of interest.

Author contributions

E.F. wrote the manuscript. N.T. performed the cytokine measurements. T.N., D.H., M.K., H.T., T.N. gave conceptual advice. All authors read and approved the final manuscript.

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