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Authors' contributions
TM drafted the manuscript and participated in its design. SF, RO, and TI participated in drafting of the manuscript and participated in its design. YI participated in the drafting of the manuscript and supplied the pathological image used for the manuscript. SY conceived of the case report, participated in drafting the manuscript and gave final approval for the version to be submitted for publication.

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Original Article

Soluble tumor necrosis factor receptor-1 in preterm infants with chronic lung disease

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Abstract ***Background***: It is clear that inflammation plays an important role in developing chronic lung disease in preterm infants. The purpose of the present study is to investigate changes of serum soluble tumor necrosis factor receptor-1 levels over time in infants with chronic lung disease.

Methods: The serum levels of soluble tumor necrosis factor receptor-1 were measured after delivery, and at 7, 14, 21 and 28 days of age in 10 infants with chronic lung disease and in 18 infants without chronic lung disease.

Results: The serum level of soluble tumor necrosis factor receptor-1 was significantly higher in infants with chronic lung disease than in infants without chronic lung disease after delivery. The differences between these two groups remained up to 28 days of age.

Conclusion: Prenatal inflammation with persistence into postnatal inflammation may be involved in the onset of chronic lung disease.

Key words chronic lung disease, fetal inflammatory response syndrome, inflammation, preterm infants, soluble tumor necrosis factor receptor-1.

The mechanisms of the onset of diseases that specifically develop in premature infants, such as chronic lung disease (CLD), retinopathy of prematurity, and periventriclar leukomalacia, are not completely understood. These diseases were thought to originate from clinical conditions that gradually form due to external factors, such as postnatal respiratory and circulation management, along with the underlying immaturity of each organ involved. However, recent studies have revealed that prenatal infection and inflammation may play important roles in the onset of these diseases.[1–6] Fetal inflammatory response syndrome (FIRS) is a systemic inflammatory response in which the fetal blood interleukin (IL)-6 level is elevated, and is seen in fetuses with premature births and preterm rupture of the membrane. FIRS is an independent risk factor for the occurrence of diseases such as CLD and necrotizing enterocolitis (NEC).[7] In addition, it has been reported that the levels of fetal blood soluble tumor necrosis factor receptor-1 (sTNFR-1) and soluble tumor necrosis factor receptor-2 (sTNFR-2) are elevated in fetuses with FIRS.[8] Many studies have reported the association between inflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor (TNF)-α and CLD, but there have never been any articles describing sTNFR-1. Actually, we have found that TNFR-1 may be easier to detect than other inflammatory cytokines, such as IL-1β and IL-6, in preterm infants (data not shown). In the present study, postnatal serum sTNFR-1 levels were measured up to 28 days of life in premature infants and the relationship between the sTNFR-1 level and the onset of CLD was examined. The purposes of this study were: (i) to investigate the relationship between the serum sTNFR-1 level at birth and the onset of CLD; and (ii) to compare the changes in serum sTNFR-1 level from birth to 28 days of age between the infants who did or did not develop CLD.

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Methods

Eighty-one preterm infants with a gestational age of less than 34 weeks, who were admitted to the neonatal intensive care unit at Yokohama City University Medical Center and Katsushika Red Cross hospital from May 2003 to March 2004, were enrolled in this study. Infants with infectious diseases such as sepsis or NEC, and those with congenital malformations were excluded. Blood samples were obtained from infants after delivery, and at 7, 14, 21 and 28 days of age. Infants in whom measurement data were missing due to clinical reasons or insufficient sample volume were excluded. Finally, data from 28 infants were analyzed. This study was approved by the ethics committee of Yokohama City University. Informed consent was obtained from the parents of all of the infants.

Table 1 Characteristics of population

	CLD group	non-CLD group	*P*-value
Number	10	18	
Gestational age (weeks)	Median 28 [min 23, max 31]	Median 30 [min 27, max 34]	0.003*
Birthweight (gram)	Median 1045 [min 584, max 1516]	Mean 1376 [min 1057, max 1794]	0.008*
Male	4 [40.0%]	5 [27.8%]	0.507**
Antenatal steroid therapy	4 [40.0%]	6 [33.3%]	0.774**
Cesarean section	6 [60.0%]	10 [55.6%]	0.82**
Chorioamnionitis	3 [30.0%]	9 [50.0%]	0.306**
Apgar score (1 min)	Median 6.5 [min 4, max 8]	Median 7.5 [min 1, max 9]	0.326*
Apgar score (5 min)	Median 7.5 [min 3, max 9]	Median 9 [min 1, max 10]	0.15*
Mechanical ventilation	7 [70.0%]	7 [38.9%]	0.055**
Immunoglobulin M (mg/dL)	Median 8.55 [min 3.0, max 198.0]	Median 9.4 [min 3, max 91.6]	0.924*
White blood cell count (/mm3)	Median 9300 [min 4200, max 37200]	Median 10795 [min 6490, max 24400]	0.649*
C-reactive protein (mg/dL)	Median 0.09 [min 0.01, max 0.95]	Median 0.02 [min 0.00, max 1.67]	0.443*

*Differences were analyzed using the Mann–Whitney *U*-test (two-tailed). **Differences were analyzed using the χ^2-test (two-tailed).

CLD was diagnosed when there were respiratory distress symptoms that required supplemental oxygen shortly after birth with continuation beyond 28 days of age. Blood samples were collected after delivery and at 7, 14, 21 and 28 days of age. The blood was immediately centrifuged, and the serum was kept at −20°C until the measurement. The serum sTNFR-1 level was measured using an enzyme-linked immunosorbent assay kit (Quantikines, R & D Systems, Minneapolis, MN, USA).

Statistical analysis

Proportions in groups were compared using the unpaired *t*-test, the χ^2-test, and the Mann–Whitney *U*-test. The groups were also compared using logistic regression analysis. A *P*-value of 0.05 and <0.05 was considered as significant.

Results

Among the 28 infants in the analysis, 10 infants developed CLD and were placed in the CLD group. The remaining 18 patients were placed in the non-CLD group. The characteristics of the CLD and non-CLD groups are summarized in Table 1. The gestational age and birthweight were significantly lower in the CLD group than in the non-CLD group ($P = 0.003$ and $P = 0.008$, respectively). There were no differences in other characteristics such as sex, the modes of delivery and Apgar scores between the CLD group and the non-CLD group. Also, there were no significant differences in the levels of inflammatory markers, such as serum level of immunoglobulin M (IgM), white blood cell count, or serum level of C-reactive protein (CRP) at birth between the two groups.

Table 2 is the result of logistic regression analysis. Infants in the CLD group were younger and smaller than those in the non-CLD group (Table 1). There was a correlation between gestational age and birthweight ($R^2 = 0.597$, data not shown), so that logistic regression analysis was performed in gestational age and sTNFR-1 level. Both gestational age and the sTNFR-1 level showed significant differences between the CLD group and the non-CLD group. This shows that the level of sTNFR-1 in cord blood may be an independent risk factor for CLD.

The serum level of sTNFR-1 decreased during the first week after birth in both the CLD and the non-CLD group. In the CLD group, the sTNFR-1 level decreased over the first 4 weeks. On the other hand, the sTNFR-1 level remained the same after day 7 in non-CLD group. At all time points (at birth, and 7, 14, 21, and 28 days of age), the sTNFR-1 level was significantly higher in the CLD group than in the non-CLD group (Fig. 1).

Discussion

The results of the present study showed that an elevated serum sTNFR-1 level in preterm infants was closely associated with the onset of CLD. In addition, the difference in sTNFR-1 level at birth between the CLD group and the non-CLD group did not disappear after birth and was still seen at 28 days of age. TNF-α is a proinflammatory cytokine produced by macrophages and lymphocytes[9–11] and its effect occurs through binding to its receptor on the surface of cells. There are two types of TNF-α receptors. Their molecular weights are 55 kDa and 75 kDa. They are called tumor necrosis factor receptor-1 (TNFR-1) and tumor necrosis factor receptor-2 (TNFR-2), respectively.[8,12,13] sTNFR-1

Table 2 The risk factor for chronic lung disease (CLD)

	CLD ($n = 10$) median (range)	non-CLD ($n = 18$) median (range)	*P*-value
Gestational age (week)	28 (23–31)	30 (27–34)	0.035
sTNFR-1 (pg/mL)	5101 (2770–9707)	2656 (1287–4462)	0.048

Logistic regression was performed. There were significant differences in both of gestational age and the sTNFR-1 level between CLD and non-CLD group. sTNFR-1, soluble tumor necrosis factor receptor-1.

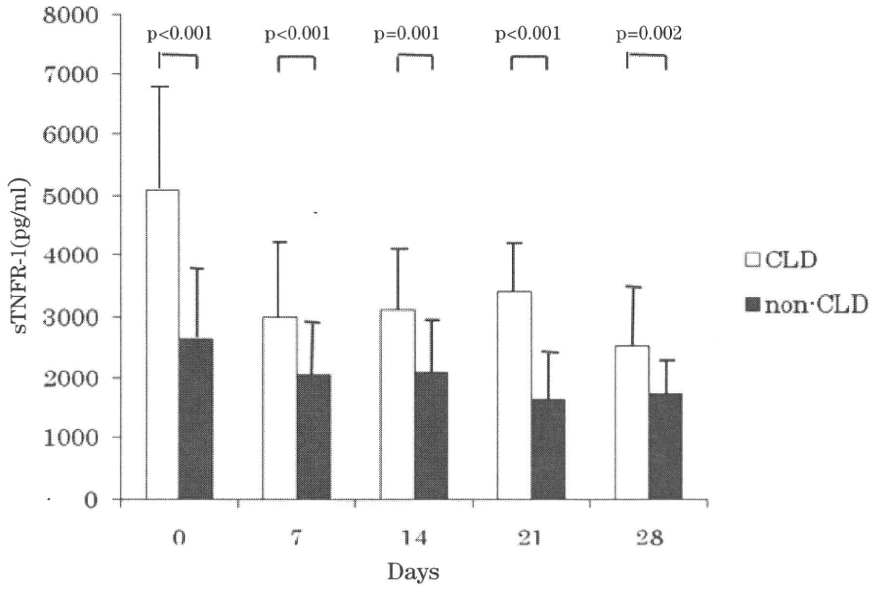

Fig. 1 Changes in the serum soluble tumor necrosis factor receptor-1 (sTNFR-1) level in premature infants over time. Bars indicate standard deviation. Statistical difference in the mean sTNFR-1 between (□) infants with chronic lung disease (CLD) and (■) infants without CLD was tested using the unpaired *t*-test.

and sTNFR-2 are fragments that are shed into the blood due to proteolytic cleavage of TNFR-1 and TNFR-2, respectively. Shedding of sTNFR-1 and sTNFR-2 increases when there are increased levels of cytokines such as TNF and IL-6.[14] An increased sTNFR-1 level is a marker of existing infection. It has been reported that the serum sTNFR-1 level is significantly increased in infants with infections.[14] As the blood sTNFR-1 level was high at birth in our CLD group, it is believed that a prenatal infection was related to the onset of CLD. At all time points (birth, and 7, 14, 21, and 28 days of age), the sTNFR-1 level was higher in the CLD group than in the non-CLD group. Shedding of sTNFR-1 may continue due to some means of stimulation in the infants after birth, because the half-life of sTNFR-1 is shorter than 2 h.[15] Due to prenatal exposure to infection, the infant's lung tissue may be vulnerable and be in a state in which an excessive inflammatory response readily occurs from minimal injury to the lung after birth.[16,17] In other words, the CLD group may have already been in a state in which sTNFR-1 is easily shed at the time of birth. Therefore, the serum sTNFR-1 level may have increased even though these patients received the same general management as that given to the non-CLD group. Cope *et al.* reported that IL-4 modulates the sTNFR-1 level.[18] However, the means by which the sTNFR-1 level is regulated is still unknown.[14] In order to explain the mechanism accounting for the difference in sTNFR-1 level that continued after birth between the CLD group and the non-CLD group, further studies are needed to determine factors that promote or inhibit shedding of sTNFR-1.

There were no direct relationships between the IgM or CRP level at birth and the onset of CLD. The reason for this is thought to be that even if infection was present, it did not reach a level that would lead to the production of IgM and CRP. Studies on the cytokine network, such as sTNFR-1, rather than inflammatory markers such as IgM or CRP, are essential in order to elucidate the relationship between infection and the onset of CLD.

The level of sTNFR may be associated with chorioamnionitis (CAM). But patients with CLD did not experience a high rate of CAM in the present study (Table 1). On the contrary, the rate of CAM was low in the CLD group. There are two possible reasons for this: first, the size of the study population in this study was small. We may be able to observe the relationship between sTNFR-1 level and CAM when we increase the number of patients. Second, even if there was an increase of sTNFR-1 level, lymphocyte infiltration is so mild that CAM might not have been observed in the histological examination.

There have been many reports on the relationship between the cytokine levels in amniotic fluid, umbilical cord blood or tracheal aspirate of premature infants and the onset of CLD. The relationship between increased levels of TNF-α, IL-1, IL-6, and IL-8 in amniotic fluid and the onset of CLD has been shown.[19] An *et al.* reported that the IL-6, IL-8 and sTNFR-1 levels in umbilical cord blood were significantly higher in infants who developed CLD.[20] Groneck *et al.* reported that the IL-1 and IL-8 levels in tracheobronchial aspirate were high in infants with CLD.[21] It has also been reported that there was no difference in the IL-6 level in lung lavage fluid after birth between CLD infants and non-CLD infants; however, IL-6 activity was significantly higher in the CLD infants.[22]

To the best of our knowledge, there has been no histological evidence that shows that TNF and TNFR are involved in developing CLD. It is stated that the sTNFR-1 level in cord blood was significantly higher in infants with CLD than infants without CLD, but the TNF-α level was not.[20] Further detailed studies

using immunological stain will be needed to elucidate the histological characteristics of TNF-α and sTNFR-1.

Cytokine levels in blood samples collected directly from neonates do not appear to have been studied previously. A literature search showed that there have only been a few reports on the change of postnatal cytokine levels in the blood.[23–26] In the present study, blood samples were collected every week from birth to 28 days of age in premature infants to measure the blood sTNFR-1 level and chronological changes could be shown. Aderka *et al.* reported that there was variation in the sTNFR-1 level among healthy adults with a range of approximately 500–1400 pg/mL.[27] Hummerich *et al.* reported that the blood sTNFR-1 levels in adults (19–49 years old) were 1630–1890 pg/mL.[28] In the present study, the median sTNFR-1 level among premature infants at 28 days of age was 2535 pg/mL in the CLD group and 1726 pg/mL in the non-CLD group. It is very interesting that the sTNFR-1 level in the non-CLD group had already declined to the same level as that in adults at 28 days of age. Menon *et al.* compared the levels of TNF-α, sTNFR-1, and sTNFR-2 in amniotic fluid between black patients and white patients during active labor and reported that TNF-α/sTNFR-1 and TNF-α/sTNFR-2 in the amniotic fluid were higher in black patients with premature births than in white patients with premature births.[29] It appears that there is a racial difference in the pathological process of preterm birth mediated by TNF-α.

In conclusion, the serum sTNFR-1 level at birth was significantly higher in the CLD group than in the non-CLD group. The serum sTNFR-1 level showed a tendency to decrease after birth, but was still higher in the CLD group at 28 days of age. Therefore, this study suggests that prenatal inflammation with persistence into postnatal inflammation is involved in the onset of CLD. Establishment of measures to reduce inflammation in preterm infants may lead to methods of prevention and treatment of CLD.

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