

CD3+/TCR $\alpha\beta$ and CD3+/TCR $\gamma\delta$ lymphocytes in full term neonates with early-onset pneumonia – influence of perinatal risk factors

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Abstract

The aim of the study was the evaluation of CD3+/TCR $\alpha\beta$ and CD3+/TCR $\gamma\delta$ lymphocytes in peripheral vein blood of full term eutrophic neonates with severe pneumonia. The study comprised of 71 neonates: 46 neonates with pneumonia and 25 healthy ones.

Methods. Immunological analysis was performed in the flow cytometer FACScan using anti-CD3, anti-TCR $\alpha\beta$ and anti-TCR $\gamma\delta$ monoclonal antibodies from Becton Dickinson.

Results. It was found that the mean percentage of lymphocytes in pneumonic neonates was significantly higher than in the controls. The mean number of these cells and the mean number and percentage of CD3+/TCR $\gamma\delta$ lymphocytes in pneumonic neonates did not differ significantly from the values of these parameters in healthy neonates. We also found that the mean number of CD3+/TCR $\gamma\delta$ in sick neonates with perinatal risk factors was significantly higher than in pneumonic neonates without perinatal risk factors

Conclusion. 1. Early-onset pneumonia is the factor significantly increasing the percentage of CD3+/TCR $\alpha\beta$ T lymphocytes in full-term neonates.

2. Increase of the number of CD3+/TCR $\alpha\beta$ T lymphocytes associated with presence of perinatal risk factors in full term pneumonic neonates provides important information about changes in cell-mediated immunity during the early neonatal period.

Key words: CD3+/TCR $\alpha\beta$ lymphocytes, CD3+/TCR $\gamma\delta$ lymphocytes, neonate, pneumonia, perinatal risk factors

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Introduction

Despite recent advances in neonatal intensive care and administration of broad-spectrum antimicrobial agents, early-onset pneumonia remains severe disease characterized by high mortality rate. Early-onset pneumonia (EOP) is caused by the infection of amniotic fluid via maternal perineum or genital tract, with clinical or subclinical chorioamnionitis. The incidence of EOP over a 41-month period in Oxford was 1,78 per 1000 live births [1]. EOP has been diagnosed at autopsy in 15-38% of stillborn and 20-32% of live born babies who died [2]. Some authors

emphasize, that the increase of mortality rate due to severe EOP is caused by weak and inappropriate immunity. Lymphoid tissue, apart from thymus is poorly developed in neonate. However, absolute number of T-cells in full-term neonates is comparable to values in older children. Although the ability of these cells to respond to various microbiological antigens is reduced, the agents can stimulate and activate proliferation and maturation of T-cells [3-5]. There is a lot of receptors on the surface of T-cell, but among them T-cell receptor (TCR) is considered to be the most characteristic. Discovery of TCR and explanation of its role in antigen recognition was made by Zinkernagel and

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Doherty, and honoured with the Nobel Prize in 1996. There is about 5×10^4 TCR on the surface of a T-cell, binding and responding to peptide antigens. TCR could also recognize and respond to lipid and glycolipid antigens. Two phenotypes of T-cell can be distinguished: TCR1 with $\gamma\delta$ chains and TCR2 with $\alpha\beta$ chain [6, 7]. The role of $\gamma\delta$ lymphocytes is still obscure. Because of characteristic location in the skin and mucosal membranes, it is assumed that they play protective role at the border between the organism and environment. Increased number of $\gamma\delta$ T-cells is found in inflamed tissues [8].

Smith et al. [9] found that the percentage of TCR1 in healthy neonates is lower in comparison with adults and cord blood and ranges 1,8%.

This study attempted to indicate the percentage and number of CD3+/TCR $\alpha\beta$ and CD3+/TCR $\gamma\delta$ lymphocytes subpopulations in peripheral vein blood of healthy full-term neonates and pneumonic, mechanically ventilated neonates as well as to investigate the influence of selected perinatal risk factors on these lymphocyte subsets.

Material and methods

The study involved 46 full-term neonates (25 male and 21 female) with the diagnosis of EOP admitted between 1996 and 2001 to the Neonatal Intensive Care Unit of the II Department of Pediatrics in Zabrze. All babies required mechanical ventilation since the 48 hours of life.

Inclusion criteria were as follows:

1. Neonates between 38 and 41 weeks of gestation at birth and of appropriate size for gestational age
2. Less than two days of age at study entry
3. No septicemia, bacteriemia (negative blood cultures in all pneumonic neonates) and congenital abnormalities
4. Symptoms of severe respiratory distress within 48 hours of life: tachypnoe, intercostals and sternal retraction, nasal flaring and cyanosis
5. Diagnosis of pneumonia confirmed by X-ray examination.

In six neonates pulmonary hypertension was diagnosed. In 28 (61%) neonates bacteria (Staphylococcus aureus in 12, Streptococcus B in 4, Klebsiella pneumoniae in 6, Haemophilus influenzae in 3, Pseudomonas aeruginosa in 2 cases irrespectively) were isolated from endotracheal aspirates and Candida albicans in one patient. In 16 neonates elevated neutrophile counts were observed in gastric aspirates. Every patient had continuous monitoring of transcutaneous PaO₂, PaCO₂ and O₂ saturation. Nineteen neonates with pneumonia required dopamine infusion. All the pneumonic neonates were treated with wide spectrum antibiotics (mainly netilmicine with ampicilin or III-rd generation cephalosporine). In 10 neonates packed platelet infusion and in 4 packed red blood cell infusions were necessary because of thrombocytopenia and anemia. Cases of hyperbilirubinemia (11 neonates) were

treated with phototherapy. Since 3-4 days of age, when oral feeding was well tolerated, enteral nutrition was started. Measurement of plasma electrolytes, glucose, calcium and total protein in all neonates were performed. Six deaths (in second week of age) due to fulminant pneumonia were recorded among the sick neonates.

The following were included to perinatal risk factors:

a) infectious factors:

- premature rupture of fetal membranes (>24 hours) in 12 cases,
- maternal infection (bacteriuria or infection of respiratory tract) in 7 cases

b) non-infectious factors:

- birth asphyxia (Apgar score<6 and umbilical cord blood pH<7,16) in 19 cases
- maternal preeclampsia (mainly high blood pressure, edema and/or proteinuria) in 8 cases
- cesarean section in 19 cases (urgent in 14, elective in 5 cases)

Healthy control subjects. The control group included 25 full-term healthy, born vaginally by healthy mothers and without signs of either infections, congenital abnormalities, hiperbilirubinemia or perinatal risk factors. All neonates had Apgar score higher than nine and were breast fed from the first day of life.

Immunophenotyping. Heparinized blood samples (1 ml) were obtained by venipuncture in pneumonic neonates between the second and the fourth day of life, immediately after admission to the NICU. In control group blood was sampled from peripheral vein at the age of 2-3 days. The samples were collected according to the consent guidelines of the medical ethics committee of the Silesian University of Medical Sciences in Katowice. Blood samples were incubated with FITC and/or PE-conjugated mouse anti-human monoclonal antibodies (MoAbs) for 30 minutes at room temperature, in the dark. Erythrocytes were lysed with fluorescence-activated cell sorter lysing solution (Becton Dickinson) according to the manufacturer's instruction. The cells were washed twice in the phosphate buffer, fixed with 0,5% paraformaldehyde. Lymphocytes were gated and for each sample 10000 gated event were analyzed with FACScan flow cytometer (Becton Dickinson) equipped with Cell Quest Software. The analysis was performed using the monoclonal antibodies specific for CD3+/TCR $\alpha\beta$ and CD3+/TCR $\gamma\delta$.

The results were presented as percentage and number expressed in giga per liter (G/l)

Statistical analysis. Mean values and standard deviations (SD) of recorded parameters were calculated. For comparison between the study group and healthy controls Mann and Whitney's U-test was applied. P values <0,05 were assumed as a statistically significant.

The study was accepted by the Local Ethical Commission for Research Studies, Silesian University of Medical Sciences in Katowice.

Results

Table 1 presents the number of leukocytes, lymphocytes and number and percentage of CD3+/TCRαβ and CD3+/TCRγδ in peripheral vein blood in all 71 full-term neonates. The mean percentage of CD3+/TCRαβ T-cells in severe pneumonic neonates ranged from 51 to 80% with mean 61,05±8,54% and was significantly higher (p=0,000045) than in healthy neonates in which mean value was 47,40±9,81% (ranged from 36 to 65%). However mean number of these lymphocytes was 2,68±1,21 (range: 1,21-6,29 G/l) in neonates with pneumonia and was similar to

healthy neonates. Absolute number of these lymphocytes in control group ranged from 1,39 to 4,83 G/l (mean 2,41±1,18)

The mean percentage of CD3+/TCRγδ in patients with pneumonia was 2,75±1,65% (range: 1,00-7,00%) and did not differ from the mean value in controls (3,53±1,21%). Similarly, the number of CD3+/TCRγδ ranging from 0,034 to 0,40 G/l (mean: 0,18±0,18 G/l) in pneumonic neonates was close to values observed in control group with mean 0,18±0,10 G/l (range: 0,07-0,35 G/l).

We have also found that perinatal risk factors did not significantly influence on the mean percentage of CD3+/TCRαβ and CD3+/TCRγδ lymphocytes and the mean number of CD3+/TCRαβ in pneumonic neonates (table 2). The mean number of CD3+/TCRγδ in sick neonates with perinatal risk factors was significantly higher than in pneumonic neonates without perinatal risk factors.

Table 1. The number of leukocytes, lymphocytes and CD3+/TCRαβ and CD3+/TCRγδ T-cells in peripheral vein blood in 71 full-term neonates

	Pneumonia	Control group	p
Leukocyte count (G/L) mean ± SD	14,24±5,61	14,08±4,30	NS
Lymphocyte count (G/L) mean ± SD	4,49±1,60	4,99±1,59	NS
CD3+/TCRαβ (%) mean ± SD min - max	61,05±8,54 51,22-80,18	47,40±9,81 36,32-65,34	p <0,000045
CD3+/TCRαβ (G/L) mean ± SD min - max	2,68±1,21 1,21-6,29	2,41±1,18 1,39-4,83	NS
CD3+/TCRγδ (%) mean ± SD min - max	2,75±1,65 1,00-7,00	3,53±1,27 1,50-5,20	NS
CD3+/TCRγδ (G/L) mean ± SD min - max	0,18±0,18 0,034-0,40	0,18±0,10 0,07-0,35	NS

Table 2. The percentage and number of CD3+/TCRαβ and CD3+/TCRγδ T-cells in 46 pneumonic neonates according to the occurrence of perinatal risk factor (PRF)

	Neonates with PRF n=29	Neonates without PRF n=17	p
CD3+/TCRαβ (%) mean ± SD min - max	60,64±9,51 51,56-80,18	61,56±7,72 51,22-72,88	NS
CD3+/TCRαβ (G/L) mean ± SD min - max	2,96±1,43 1,59-6,29	2,55 ± 0,90 1,21-3,70	NS
CD3+/TCRγδ (%) mean ± SD min - max	3,27±1,90 1,0-7,0	2,11±1,05 2,0-6,0	NS
CD3+/TCRγδ (G/L) mean ± SD min - max	0,22±0,21 0,05-0,96	0,13±0,11 0,03-0,40	p<0,05

Discussion

Our study shows that the percentage of CD3+/TCR $\alpha\beta$ and CD3+/TCR $\gamma\delta$ lymphocyte subpopulations may vary widely and rapidly as a result of severe early-onset pneumonia. We noted immediate influence of organ infection without bacteriemia on the CD3+/TCR $\alpha\beta$ lymphocytes in full-term neonates. In pneumonic infants, independently of perinatal risk factors significant increase of the percentage of those cells was noted.

The increase of percentage of CD3+/TCR $\alpha\beta$ seems to have special significance. The role of TCR $\alpha\beta$ lymphocytes subpopulation in infection response was not yet explained, but it is known, that more than 90% of T lymphocytes in peripheral blood have $\alpha\beta$ receptors. A number of authors conclude that mothers health status and perinatal risk factors should be taken into account when assessing the immunological system function in neonates [10-12]. In our study neonates had no history of mother diabetes, viral and chlamydia infections but in 63% of severely sick neonates perinatal risk factors were present. Those risk factors, however, had no significant influence on the percentage and number of lymphocyte CD3+/TCR $\alpha\beta$ and percentage of CD3+/TCR $\gamma\delta$ subpopulation. Only the mean number of CD3+/TCR $\gamma\delta$ were significantly higher in pneumonic full-term neonates with perinatal risk factors than in babies without these risk. According to findings of Economou et al. [13] increase of blood catecholamins and cortisol occurring during the high risk delivery causing hypoxia could change neonatal immune system, especially T lymphocyte subpopulations. Result of the study and reported data allow us to assume that mature neonate in spite of immunological immaturity activates cellular mechanisms during contact with infectious antigens. This conclusion can be supported by the observation of quantitative changes of cellular receptors such as antigen binding to CD3+ lymphocyte receptors [14, 15]. These processes are complex and factors associated with it can initiate rapid reaction on a molecular level. After binding an antigen by TCR, tyrosine kinase reaction, activation of signal transducing pathways and transcription of appropriate genes starts. However, signal transducing from TCR does not always cause lymphocyte activation, this may cause anergy or can even lead to lymphocyte apoptosis. It is assumed that such phenomenon may be essential in pathogenesis and outcome of infections in neonates, in whom immunological immaturity favours susceptibility to infections. Our study shows that changes in the number of CD3+/TCR $\alpha\beta$ and CD3+/TCR $\gamma\delta$ lymphocytes are not consistent with the changes in their percentage values. According to Comans-Bitter et al. percentage has limited value and results should be rather interpreted on the basis of the number of T lymphocyte subpopulations [16]. Future studies are required not only to explain the association between of severe pneumonia and cell-mediated immunity in the first week of age but also the duration of these defects after delivery.

Conclusion

1. Early-onset pneumonia is the factor significantly increasing the percentage of CD3+/TCR $\alpha\beta$ T lymphocytes in full-term neonates.
2. Increase of the number of CD3+/TCR $\alpha\beta$ T lymphocytes associated with presence of perinatal risk factors in full term pneumonic neonates provides important information about changes in cell-mediated immunity during the early neonatal period.

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