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Fetal Cellular Immunity in Hemolytic Disease During Rh Immunization in the Second Trimester of Pregnancy

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Annotation: Purpose: The article presents the results of a study of cellular immunity indices in 20 fetuses with hemolytic disease in the second trimester of pregnancy. Materials and methods: studies were conducted on 20 umbilical cord blood of fetuses with hemolytic disease, which were taken by transabdominal cordocentesis from pregnant women with Rh-immunization from 24 to 28 weeks of gestation at the Republican Perinatal Center in 2024. All immunological studies were carried out at the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan.

Results: the number of CD3+ T-lymphocytes, CD4+ T-helper cells and CD20+ B-lymphocytes in fetuses with hemolytic disease was significantly reduced by 2.23, 2.3 and 2 times compared to the control group; the number of CD8+ lymphocytes of cytotoxic cells and CD16+ killer lymphocytes were significantly increased by 1.3 times and 3.6 times compared to the control group;

Conclusion: In hemolytic disease, the synthesis of T-lymphocytes by the fetus increases, primarily due to the general pool of CD3+, CD4+ T-helper cells and CD20+ B-lymphocytes, which naturally contributes to an increase in CD8+ cytotoxic cells and CD16+ T -killer cells, which are the result of the fetal immunological response.

Keywords: Rh-immunization, hemolytic disease of the fetus, T-lymphocytes CD3+, CD4+, CD8+, CD16+, CD20+ immunoregulatory index, II-trimester, B-lymphocytes.

Introduction: The introduction of anti-Rhesus immunoglobulin D immunoprophylaxis has significantly reduced the incidence of maternal alloimmunization to the D antigen [1]. Most guidelines for intrauterine blood transfusion focus on the prevention of alloimmunization to the D antigen [2]. However, antibodies to non-D minor antigens are present in 1.5% to 2.5% of all pregnancies [3]. Alloimmunization to the c antigen is considered the second most dangerous form of alloimmunization causing hemolytic disease of the fetus and newborn (HDFN) after alloimmunization to the D antigen [4].

There are few obstetric guidelines that address the nuances of selecting blood components for intrauterine transfusion in the context of maternal Rh immunization to minor antigens [5].

In recent years, researchers have paid more attention to immunological and immunogenetic methods for diagnosing hemolytic disease of the fetus, in particular, great attention is paid to predicting the severity. In the pathogenesis of Rh sensitization, that is, active antibody formation, disorders in the fetal immune system play an important role. According to some studies on the cytokine status of the fetus with hemolytic disease, there are reduced values of IL-8, which is a marker of deficiency of chemokine activation of innate immune cells [6].

Successful prenatal management with plasmapheresis and/or intravenous immunoglobulin in severe red cell alloimmunization has been reported recently [7]. These noninvasive methods are used to avoid or delay invasive intrauterine, intravascular blood transfusion to the fetus [8]. The principle of plasmapheresis in Rh immunization is to remove maternal alloantibodies , but antibody titers are restored after plasmapheresis; however, the rebound effect is suppressed to varying degrees if plasmapheresis is followed by intravenous immunoglobulin [9,10].

In connection with the study of the humoral link of immunity, and the identification of the activity of humoral factors of immunity, we were presented with the opportunity, and a scientific interest arose, to

study the main cellular values of immunity, directly forming the immune response in response to intrauterine sensitization.

The aim of the study was to evaluate cellular immunity factors such as the main T-lymphocytes CD 3+, T-helpers CD 4+, T-suppressors CD 8+, T-killers CD 16+ and B-lymphocytes CD 20+, and also to determine the immunoregulatory index, i.e. the ratio of CD4+/CD8+ T-lymphocytes in the umbilical cord blood of fetuses with hemolytic disease caused by rhesus conflict in pregnant women in the second trimester.

Research materials: Immunological studies were conducted on 20 samples of umbilical cord blood from fetuses with hemolytic disease, which were taken by transabdominal cordocentesis in pregnant women in the second trimester with Rh immunization in the Republican Perinatal Center for 2024. In order to compare the results of the study in the second trimester in fetuses, we used data from a reliable number of 10 fetuses, which also underwent fetal studies such as transabdominal cordocentesis for diagnostic purposes in case of suspicion of various developmental anomalies, but these congenital malformations were not confirmed genetically. Therefore, they were taken as a control group.

Immunological research methods: Research of the immune status of the fetus in umbilical cord blood was conducted in the laboratory of fundamental immunology of the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan . Determination of cellular immunity included CD 3+, CD 4+, CD 8+, CD 20+, CD 16+/56+ and was carried out using monoclonal antibodies from BD (USA) by flow cytometry (BD Accuracy C 6).

The immunoregulatory index (IRI), which is the ratio of the number of CD4+ T-helpers to the number of CD8+ T-lymphocytes, was calculated manually. Normally, the IRI in healthy children and adults is higher than one.

Statistical processing of results The results were analyzed using parametric and nonparametric analysis methods. The accumulation, adjustment, systematization of the initial information and visualization of the obtained results were carried out in Microsoft Office Excel 2018 spreadsheets. Statistical analysis was performed using IBM SPSS Statistics v.26 (developer - IBM Corporation). When comparing average values in normally distributed sets of quantitative data, Student's t-test was calculated. The obtained values of Student's t-test were assessed by comparison with critical values. Differences in indicators were considered statistically significant at a significance level of $p < 0.05$.

Results and discussion: We conducted studies of adaptive or specific cellular immunity of the fetus. As for the fetus, we studied umbilical cord blood from the vein of a fetus with hemolytic disease at 24 to 28 weeks of gestation in women with Rh-conflict.

Table 1 presents the results of the study of adaptive cellular immunity of the fetus in the second trimester of pregnancy.

Table-1. Main cellular parameters of fetal immunity in the second trimester of pregnancy (M±m , %)

Parameters	Main group (n=20 fruits)	Control group (n = 10 fruits)
CD3+, %	19.82±1.85 *	44.07±8.77
CD4+, %	16.92±1.08 *	38.12±2.23
CD8+, %	17.12±0.42 *	13.55±2.53
CD4+/CD8+, IRI	0.8±0.02 *	1.85 ±0.24
CD20+, %	8.65 ±0.55 *	15.35±3.71
CD16+ /CD56+, % %	1 3, 4 2 ±1, 35 *	3.82±0.66

Note: * – significance of differences with the control group $p < 0.05$

The analysis showed that the total number of CD 3+ T-lymphocytes was significantly reduced compared to the control values. Thus, the average content of T-lymphocytes was 19.82±1.85%, which was 2.23 times lower than the control values. This most likely indicates a decrease in the proliferation of T-lymphocytes, which can affect the decrease in the overall cellular immunity of the fetus in HD. It is known that the phenotypic markers of T-lymphocytes include CD3+, CD4+, CD8+ receptors. It has been shown that the initiation and regulation of the effectiveness of the immune response is largely determined by the specific antigen of T-lymphocytes , which are responsible for this function and are antigen recognition receptors - TCR. It is known that the degree of surface expression of CD3+ receptors on the T-lymphocyte membrane reflects its transmission function and allows identifying the total number of T-lymphocytes.

Further analysis showed that the total number of CD 4+ helper/inducer T-lymphocytes was also significantly reduced compared to the control values. Thus, the average content of helper/inducer T-lymphocytes was 16.92±1.08%, which was 2.3 times lower than the control values. This indicates not only a decrease in T-lymphocyte proliferation, but also a reduced immune response of the fetus to various antigens. The CD 4+ T-cell response is an important mechanism of body defense, since CD 4+ T-helpers stimulate the production of antibodies by B-lymphocytes and activate CD 8+ T-lymphocytes, which are quite specific.

Further analysis showed that the total number of T-cytotoxic cells CD 8+ were significantly increased compared to the control values. Thus, the average content of T-cytotoxic lymphocytes was 17.12±0.42%, which was 1.3 times higher than the control values. This indicates not only an increase in the proliferation of T-cytotoxic lymphocytes, but also an increased load on the immune system as a whole. It is known that with GB, cytotoxic cells are activated and have a destructive effect on the body.

It has been established that CD4+ T-helpers are functionally divided into two types of helper lymphocytes: the so-called T-helpers type 1 (TH1) and type 2 (TH2), with TH1 producing cytokines of the cellular immune response, and TH2 producing cytokines of the humoral immune response, which is important in assessing the immunoregulatory properties of the body, which characterizes the functional activity of immunocompetent cells (ICC) in pathological conditions. Cytotoxic CD8+ T-lymphocytes play an important role in the pathogenesis of many diseases. The function of these cells is to recognize antigens on the cell surface in complex with MHC class 1 molecules. Since they are present on virtually all nucleated cells of the body, any cell carrying MHC class 1 molecules in complex with an antigenic peptide can activate a clone of cytotoxic T-lymphocytes. The biological role of this activation is the elimination of mutant or destroyed cells. CD8+ T lymphocytes play a major role in elimination, which is due, on the one hand, to their ability to cause the death of mutant cells expressing the corresponding peptides presented by MHC class 1 molecules, and on the other hand, to the ability to secrete various types of cytokines with anti-inflammatory activity.

The immunoregulatory index (IRI), which is the ratio of the number of CD4+ T-helpers/inducers to the number of CD8+ T-lymphocytes, is of significant importance in pathologies . Normally, the IRI in healthy children and adults is higher than one. The data obtained are presented in Table 1. It is obvious that the suppression of IRI is due to a decrease in T-helpers/inducers and an increase in T-cytotoxic lymphocytes, which leads to a decrease in IRI ($p < 0.05$). The analysis showed that the IRI, or rather the immunoregulatory index, was reliably suppressed compared to the control values. Thus, the average content of IRI was 0.8 ± 0.02, which is 2.4 times lower than the control values. Most likely, the suppression of IRI also reflects the formed cellular immunodeficiency.

Further analysis showed that the number of B-lymphocytes CD 20+ was significantly reduced compared to the control values. Thus, the average content of B-lymphocytes was 8.65±0.55%, which was almost 2 times lower than the control values. This indicates not only the suppression of the systemic immunity of the fetus, but also the load on the bone marrow, which is unable to produce B-lymphocytes of the fetus.

Analysis of the killer activity of fetal immunity showed that the number of killer lymphocytes CD 16+/56+ was significantly increased compared to the control values. Thus, the average content of killer cells was 13.42 ± 1.35%, which was almost 3.6 times higher than the control values. It is known that natural killer cells (NK) are the third population of lymphocytes that ensure the maintenance of genetic homeostasis, which are phenotypically and functionally significantly different from T- and B-lymphocytes.

Conclusions:

CD 3+ T-lymphocytes by the fetus decreases by 2.23 times, as well as CD 4+ T-helpers by 2.3 times, which naturally also contributes to a decrease in CD 20+ B-lymphocytes, which are the result of the immunological response of the fetus.

2. High levels of both cytotoxic CD 8+ T-lymphocytes and T-killers CD 16+ are evidence that the cytotoxic process is active in the fetus's body and may result in the formation of a profound immunodeficiency in the fetus. As mentioned above, the cytotoxic type of immune response.

3. The immunoregulatory index (IRI), which is the ratio of the number of CD4+ T-helpers/inducers to the number of CD8+ T-lymphocytes, is of significant importance in pathologies. Suppression of IRI in fetuses by 2.4 times compared to the control group is due to a decrease in T-helpers and an increase in T-cytotoxic lymphocytes, which leads to a decrease in IRI ($p < 0.05$). A decrease in IRI is an important criterion for the depth of the T-cell immunodeficiency state.

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