IMMUNOMODULATORY EFFECTS OF THE PLACENTAL CONDITIONED MEDIUM AND HLA-DR-DEPENDENT MECHANISMS IN CARRIERS OF THE HLA-DRB1*01:01 ALLELES WITH PREECLAMPSIA

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Preeclampsia (PE) is considered as one of the most severe pregnancy complications, an important role in pathogenesis of which is played by immunogenetic factors. The study aimed to assess the contribution of HLA-DR-dependent mechanisms to the immune response regulation in PE associated with the HLA-DRB1*01:01 allele carrier state in an *in vitro* experimental model. A comparative experimental *in vitro* study was conducted. Patients with PE (n = 7), who were HLA-DRB1*01:01 carriers, and women with the normal pregnancy course (NP; n = 10), who were not HLA-DRB1*01:01, -DRB1*04:01, -DRB1*10:01 carriers, were enrolled. The $CD14^+$ and $CD4^+$ cells, as well as placental tissue samples, from which the placental conditioned medium (PCM) was derived, were obtained from peripheral blood samples. The $CD14^+$ and $CD4^+$ cells were co-cultured in both groups. PCM was added to the culture to model the effects of placental factors. In the PE group, HLA-DR was blocked with the L243 monoclonal antibody; the IgG2a isotype antibody was used in the NP group. The share of Treg-cells in the cultures was determined by flow cytometry; the IL17, IFN-γ, TNFα, IL10, and IL4 cocentrations were determined by ELISA. The direct $CD14^+$ and $CD4^+$ co-culture revealed no intergroup differences in the share of Treg and cytokine concentrations. The PCM supplementation in the PE group resulted in the decreased share of Treg and IL10 levels amid increasing IL17, TNFα, and IFN-γ. In the NP group, on the contrary, the increase in Treg counts, IL10 and IL4 levels, along with the decrease in IFN-γ levels was reported. The HLA-DR blockage in PE was associated with the decrease in IL17 and IFN-γ levels. The findings demonstrate the potential significance of placental factors and maternal immunogenetic features in the immune response regulation in PE.

Keywords: preeclampsia, normal pregnancy course, Th1/Th2/Th17/Treg axis, HLA-DRB1*01:01 allele, placental factors, *in vitro*

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Compliance with ethical standards: the study was approved by the Ethics Committee of the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology (protocol No. 11 dated November 11, 2021).

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ИММУНОМОДУЛИРУЮЩИЕ ЭФФЕКТЫ ПЛАЦЕНТАРНОЙ КОНДИЦИОНИРОВАННОЙ СРЕДЫ И HLA-DR-ЗАВИСИМЫХ МЕХАНИЗМОВ ПРИ ПРЕЭКЛАМПСИИ У НОСИТЕЛЕЙ АЛЛЕЛЯ HLA-DRB1*01:01

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Преэклампсию (ПЭ) рассматривают как одно из наиболее тяжелых осложнений беременности, в патогенезе которого существенную роль играют иммуногенетические факторы. Цель работы — оценить вклад HLA-DR-зависимых механизмов в регуляции иммунного ответа при ПЭ, ассоциированной с носительством аллеля HLA-DRB1*01:01 в экспериментальной модели *in vitro*. Выполнено экспериментальное сравнительное *in vitro* исследование. Включены пациентки с ПЭ (n = 7), являющиеся носителями HLA-DRB1*01:01, и женщины с физиологическим течением беременности (ФБ; n = 10), не являющиеся носительницами HLA-DRB1*01:01, -DRB1*04:01, -DRB1*10:01. Из образцов периферической крови выделяли клетки $CD14^+$ и $CD4^+$, а также образцы плацентарной ткани, из которых получали плацентарную кондиционированную среду (ПКС). В обеих группах осуществляли ко-культивирование клеток $CD14^+$ и $CD4^+$. Для моделирования влияния плацентарных факторов в культуру добавляли ПКС. В группе ПЭ проводили блокирование HLA-DR моноклональными антителами L243, в группе ФБ использовали изотипические антитела IgG2a. Долю Treg-клеток в культурах определяли методом проточной цитометрии, концентрации IL17, IFNγ, TNFα, IL10 и IL4 — методом ИФА. При прямом ко-культивировании клеток $CD14^+$ и $CD4^+$ различий между группами по доле Treg и концентрации цитокинов не обнаружено. Добавление ПКС в группе ПЭ приводило к снижению доли Treg и уровня IL10 на фоне повышения IL17, TNFα и IFN-γ. В группе ФБ, напротив, отмечалось повышение числа Treg, IL10 и IL4 с одновременным снижением IFN-γ. Блокирование HLA-DR при ПЭ сопровождалось снижением IL17 и IFN-γ. Полученные результаты демонстрируют потенциальную значимость плацентарных факторов и иммуногенетических особенностей матери в регуляции иммунного ответа при ПЭ.

Ключевые слова: преэклампсия, физиологическое течение беременности, ось Th1/Th2/Th17/Treg, аллель HLA-DRB1*01:01, плацентарные факторы, *in vitro*

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Preeclampsia (PE) is still one of the most dangerous pregnancy complications resulting in the high maternal and perinatal morbidity and mortality all over the world [1]. Despite the fact that the precise mechanisms underlying the PE development are still unknown, the findings of numerous studies strongly suggest the important role of the impaired immune regulation [2, 3]. It is believed that the impaired development of tolerance to paternal/fetal antigens results in the maternal-fetal interface immune homeostasis shift towards pro-inflammatory status [4]. In PE, imbalance between the effector T-helper (Th) subpopulations (Th1, Th2, Th17) and regulatory T cells (Treg) determining the formation of tolerogenic environment at the maternal-fetal interface represents on the key immune dysregulation mechanisms [5].

It has been shown that the development of PE is associated with the accumulation of the Th1 and Th17 cells producing pro-inflammatory cytokines, including interleukins (IL1, IL6, IL17), tumor necrosis factor — (TNFα), interferon — (IFN-γ) being the key inflammation modulators, in peripheral blood and the placental tissue. When assessing the cytokine profile in PE, the decrease in the concentrations of the IL4 and IL10 anti-inflammatory molecules produced by the Th2 and Treg cells also attracts attention [6–8]. The decrease in the counts and functional activity of Treg, which possess pronounced immunoregulatory properties and play a key role in providing immunological protection against the attack by the mother's immune system to the semi-allogeneic fetus, is an important component of PE [9]. A significant contribution of the immune tolerance impairment is confirmed by the cumulative data suggesting the increase in the Th17/Treg ration in women with PE [10].

Despite significant advances in understanding the PE immunopathogenesis, the question about the triggers that initiate immune dysregulation remains open. It is assumed that the altered trophoblast secretory profile contributes greatly to the development of the innate and adaptive immunity disturbances in PE [11]. The findings of many studies suggest changes in the composition and concentration of fetoplacental proteins, cytokines, chemokines, growth factors, extracellular vesicles, and other placental secretome components at the maternal-fetal [12–17]. These bioactive molecules can have both direct and indirect effects on various populations of immunocompetent cells, modulating their phenotypic and finctional characteristics ad thereby determining the immune response direction in PE.

Genes of the human leukocyte antigen (HLA) system, which play an important role in regulating the recognition of foreign agents and shaping immunological responses, are other important candidates for the search for pathogenetically substantiated associations [18]. The HLA system extremely high polymorphism results in the structural diversity of its molecules that differ in their capability of binding and presenting antigens, which, in turn, determines the specifics and severity of the subsequent T-cell immune responses. A number of papers show the relationship between the woman's HLA-DRB1 locus allele (DRB1*01:01, DRB1*04:01 or DRB1*10:01), encoding the shared epitope, a certain amino acid sequence, carrier state and reproductive and obstetric disorders, including PE [19–22]. The findings of the recent study suggest a positive association of the HLA-DRB1*01:01 allelic variant with the risk of PE [23].

Based on the current ideas about the role of HLA class II molecules in the T-cell immune response regulation it can be assumed that the HLA-DRB1*01:01 allele carrier state can be associated with the features of the placental antigen presentation. Potential differences in the repertoire of peptides presented can affect the $CD4^+$ T cell activation pattern, which contributes to the immune response preferential polarization in the direction of Th1/Th17 and the decrease in the immunoregulatory Treg cell induction. The above alterations can be probably considered as a mechanism inderlying the immune homeostasis pro-inflammatory shift in PE.

The study aimed to assess the contribution of HLA-DR-dependent mechanisms in the immune response regulation in PE associated with the HLA-DRB1*01:01 allele carrier state in the in vitro experimental model.

METHODS

Research design

The reported *in vitro* experimental study was focused in assessing the changes in Treg cell counts and pro- and anti-inflammatory cytokine concentrations during stimulation of naïve $CD4^+$ T cells by the antigen-presenting cells expressing certain HLA class II allelic variants, as well as placental factors. The research design is provided in Fig. 1.

The placental tissue and peripheral blood samples of patients with PE (index group, $n = 7$) and normal pregnancy or NP (comparison group, $n = 10$) were obtained from the obstetric departments of the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology. The patients enrolled were matched by age, comorbidities, and major risk factors.

The emergence of PE clinical symptoms in the index group was reported on average at 34.5 (31.25–36.75) weeks. As for severity, moderate PE was diagnosed in 6 (85.7%) patients, severe in 1 (14.3%) patient. The average delivery time was 38.2 (36.0; 39.96) weeks in the index group and 40 (39.18; 40.54) weeks in the comparison group. Preterm delivery was reported in 2 (28.6%) patients with PE.

Inclusion criteria for the index group: women aged 18–45; singleton pregnancy; ongoing pregnancy complicated by PE; HLA-DRB1*01:01 allele carrier state confirmed by polymerase chain reaction (PCR); informed consent to take part in the study.

Inclusion criteria for the comparison group: women aged 18–45; singleton pregnancy; ongoing normal pregnancy; no DRB1*01:01, DRB1*04:01 or DRB1*10:01 allele carrier state confirmed by PCR; informed consent to take part in the study.

Exclusion criteria: women under 18 and over 45 years of age; multiple pregnancy; pregnancy resulting from assisted reproductive technologies; severe extragenital disorder; chronic infection, cancer, or systemic autoimmune disorder; refusal to take part in the study.

Obtaining the placental conditioned medium

The placental tissue samples were collected immediately after delivery. Fragments of the villous chorion from the central part of the placenta were washed with sterile phosphate-buffered saline supplemented with 5% penicillin/streptomycin and mechanically grinded. The resulting tissue mass was cultured in 6-well culture plates (Wuxi NEST Biotechnology. Ltd., China) with added 3 mL of the ImmunoCultTM-XF medium (StemCell Technologies, Canada) for 24 h in the environment of the CO_2 incubator. The placental conditioned medium (PCM) was collected within 24 h. The PCM containing a broad spectrum of placental bioactive molecules, including cytokines, chemokines, growth factors, placental proteins, extracellular vesicles, and damage-associated molecular patterns (DAMP), was used as an integrated experimental model of placental

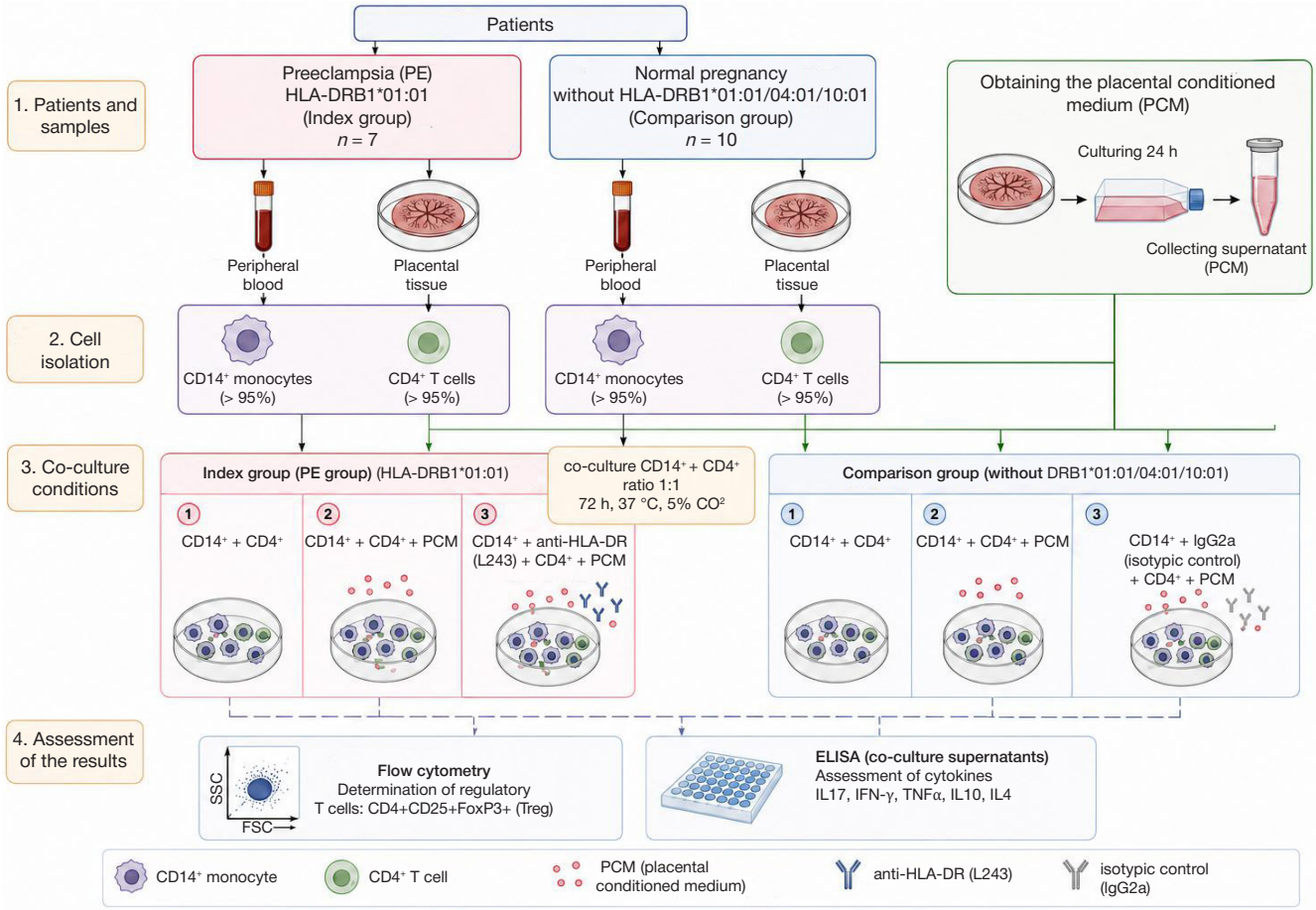

Fig. 1. Research design. CD14⁺ monocytes and CD4⁺ T cells were isolated from peripheral blood of pregnant women. The placental conditioned medium (PCM) was obtained by culturing placental explants for 24 h. Cells were co-cultured under various experimental conditions with subsequent assessment of the CD4⁺CD25⁺FoxP3⁺ Treg cell counts by flow cytometry and the cytokine concentrations in supernatants by ELISA

secretome showing the combined effects of placental factors on the maternal immune system cells.

Isolation of CD14⁺ monocytes and CD4⁺ T cells from peripheral blood

The whole peripheral blood samples were collected on the next day after delivery in the test tubes with the K3EDTA anticoagulant (Greiner Bio One, Austria).

The CD14⁺ and CD4⁺ cells were isolated from the peripheral blood mononuclear cell (PBMC) fraction by magnetic separation (positive selection) using the Human CD14⁺ Cell Separation Kit and Human CD14⁺ Cell Separation Kit (RWD Life Science, China) in accordance with the manufacturers' instructions. The resulting population purity was controlled by laser flow cytometry based on the expression of typical molecules and the presence of linear markers of other cell populations. Populations with the purity values of at least 95% were used in the study.

Cell co-culture conditions

In the first series of experiments, the CD14⁺ monocytes isolated (2×10^5 cells) were cultured with CD4⁺ T cells (2×10^5 cells), 1 : 1, in 24-well culture plates (Wuxi NEST Biotechnology. Ltd., China) with added 1 mL of the RPMI-1640 medium (PanEco, Russia) containing the 10% fetal bovine serum (FBS) (Biowest, France) in both groups.

To reproduce the T cell activation conditions *in vitro*, the ImmunoCult™ Human CD3/CD28 T Cell Activator particles (StemCell Technologies, Canada) at a concentration of 25 μg/mL were used, ensuring signal transmission through the TCR complex and costimulatory receptor CD28, essential for full-fledged activation and functional response of T cells. The cells were co-cultured for 3 days in the environment of the CO_2 incubator.

In the second series of experiments, the co-culture system was added 100 μL of the PCM obtained from the same patients to stimulate the MHC II-dependent CD4⁺ T cell activation.

In the third series of experiments, the CD14⁺ monicytes obtained from patients of the index group were pre-incubated with the purified monoclonal antibody against human HLA-DR человека (clone L243; Thermo Fisher Scientific, USA) at a concentration of 10 μg/mL for 60 min before co-culture with the autologous CD4+ T cells in order to block the MHC II/HLA-DR-dependent antigen presentation in the control series of experiments. Antibodies were present in the culture throughout the stimulation period. In control experiments in the comparison group, Mouse IgG2a (GeneTex, USA) at the same concentration were used as an isotypic control. The isotypic control was used to rule out non-specific effects of immunoglobulins on the cell functional activity.

Flow cytometry

The following monoclonal antibodies were used to identify Treg cell subpopulations: APC anti-human CD4 (clone RPA-T4), PE anti-human CD25 (clone BC96), and FITC anti-human FoxP3 (clone 206D). Membrane proteins were stained for 30 min at a temperature of 4 °C. Intracellular proteins were stained for 60 min at 4 °C after treatment with the commercially available

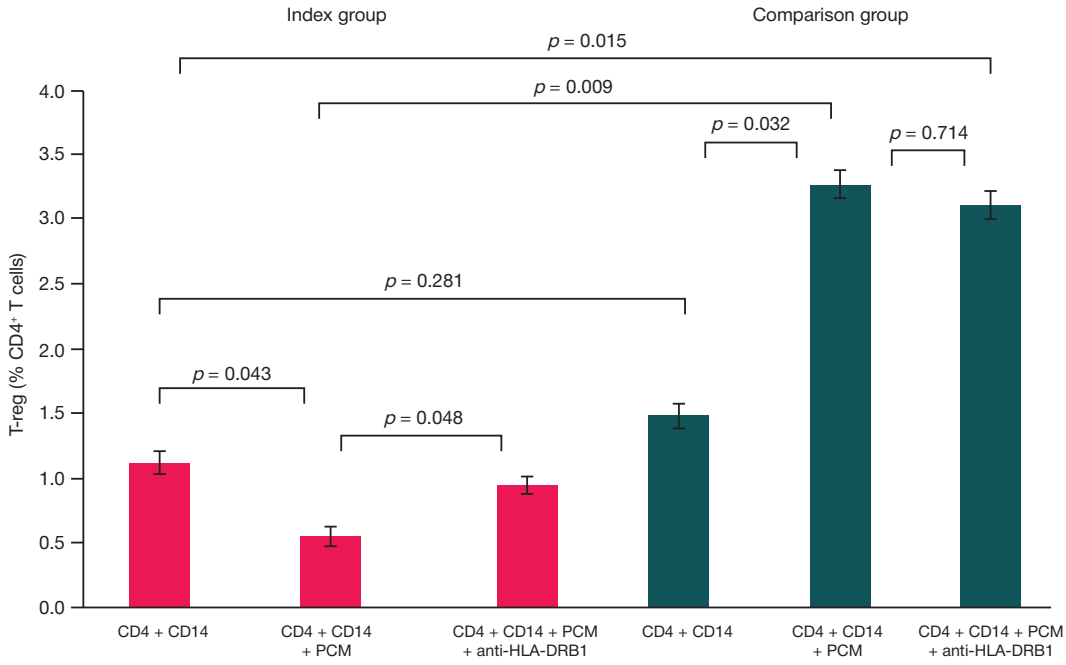

Fig. 2. Percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells in the CD4⁺ T cell population under various co-culture conditions in the index group and comparison group. Data are presented as Me (Q_1–Q_3). The nonparametric Mann–Whitney U-test was used to compare independent groups; the Wilcoxon test for related samples was used for intragroup comparisons

reagents for fixation and permeabilization from the True-Nuclear™ Transcription Factor Buffer Set (Biolegend, USA). All flow cytometry measurements were performed using the BD FACSCalibur system (BD Biosciences, USA).

ELISA

The cytokine production was qualtified by sandwich ELISA. The IL17, IFN-γ, TNFα, IL10, and IL4 concentrations in the media were quantified using appropriate commercially available test systems in accordance with the manufacturer's instructions (VectorBest, RF).

Statistical data processing

Statustical analysis of the data was conducted in R (v. 4.1.3). The quantitative variable distribution was first evaluated using the Shapiro–Wilk test and visual analysis. Considering the small sample size and non-normal distribution, the quantitative data were presented as the median (Me) and interquartile range (Q_1–Q_3). The nonparametric Mann–Whitney *U*-test was used to compare independent groups. The Wilcoxon test for related samples was used to assess the differences in culture conditions within the same group (paired observations). The differences were considered significant at $p < 0.05$.

RESULTS

Assessment of CD4⁺CD25⁺FoxP3⁺Treg cell counts

The results of assessing the CD4⁺CD25⁺Foxp3⁺ Treg cell counts under various co-culture conditions are provided in Fig. 2.

The direct CD14⁺ monocyte and CD4⁺ T cell co-culture revealed no significant differences in the T-cell response pattern in the studied groups. The percentage of Treg cells in the CD4⁺ T cell population was 1.12% in the index group and 1.49% in the comparison group ($p = 0.281$). After adding the PCM, relative Treg cell counts in the index group decreased to 0.53% (p = 0.043); in the comparison group, these, on the contrary, increased to 3.26% ($p = 0.032$). These data suggest multidirectional modulation of the T-cell response by placental factors in PE and NP ($p = 0.009$). Adding the anti-HLA-DRB1 antibody to the co-culture system in the index group changed the distribution of cells by CD25/Foxp3 and led to the significant increase in the percentage of Treg cells compared to the CD14⁺+CD4⁺+PCM conditions ($p = 0.048$). Adding the isotypic control to the co-culture in the comparison group was accompanied by the decrease in relative Treg cell counts, but the difference was non-significant ($p = 0.714$).

Assessing the cytokine profile features

Fig. 3 shows the results of assessing pro- and anti-inflammatory cytokines under various co-culture conditions.

The CD14⁺ monocyte and CD4⁺ T cell co-culture in the index group was characterized by an upward trend of the levels of pro-inflammatory cytokines IL17 ($p = 0.071$), TNFα ($p = 0.128$), IFN-γ ($p = 0.083$) and downward trend of the concentrations of anti-inflammatory ones — IL10 ($p = 0.512$) and IL4 ($p = 0.603$) relative to the similar experiment in the comparison group. In the index group, adding the PCM to the immune cell co-culture led to the significant increase in the concentrations of IL17 ($p = 0.032$), TNFα ($p = 0.038$), IFN-γ ($p = 0.027$) amid the decrease in IL10 levels ($p = 0.044$), which could reflect the immune response shift towards pro-inflammatory phenotype in PE. In contast, in the CD14⁺+CD4⁺+PCM experimental conditions, the comparison group showed a significant increase in the concentrations of immunoregulatory molecules IL10 ($p = 0.049$), IL4 ($p = 0.039$) along with the decrease in the levels of the IFN-γ inflammatory cytokine ($p = 0.046$). The pronounced intergroup differences reported for all studied cytokines after adding placental factors attract attention, which, together with the multidirectional nature of changes, suggest the key role in the immune response modulation in pregnancy. The HLA-DRB1*01:01 blockage in the index group led to the significant decrease in the IL17 and IFN-γ levels, which suggests the MHC II-dependent mechanism of their production. At the same time, adding the control IgG in the comparison group had

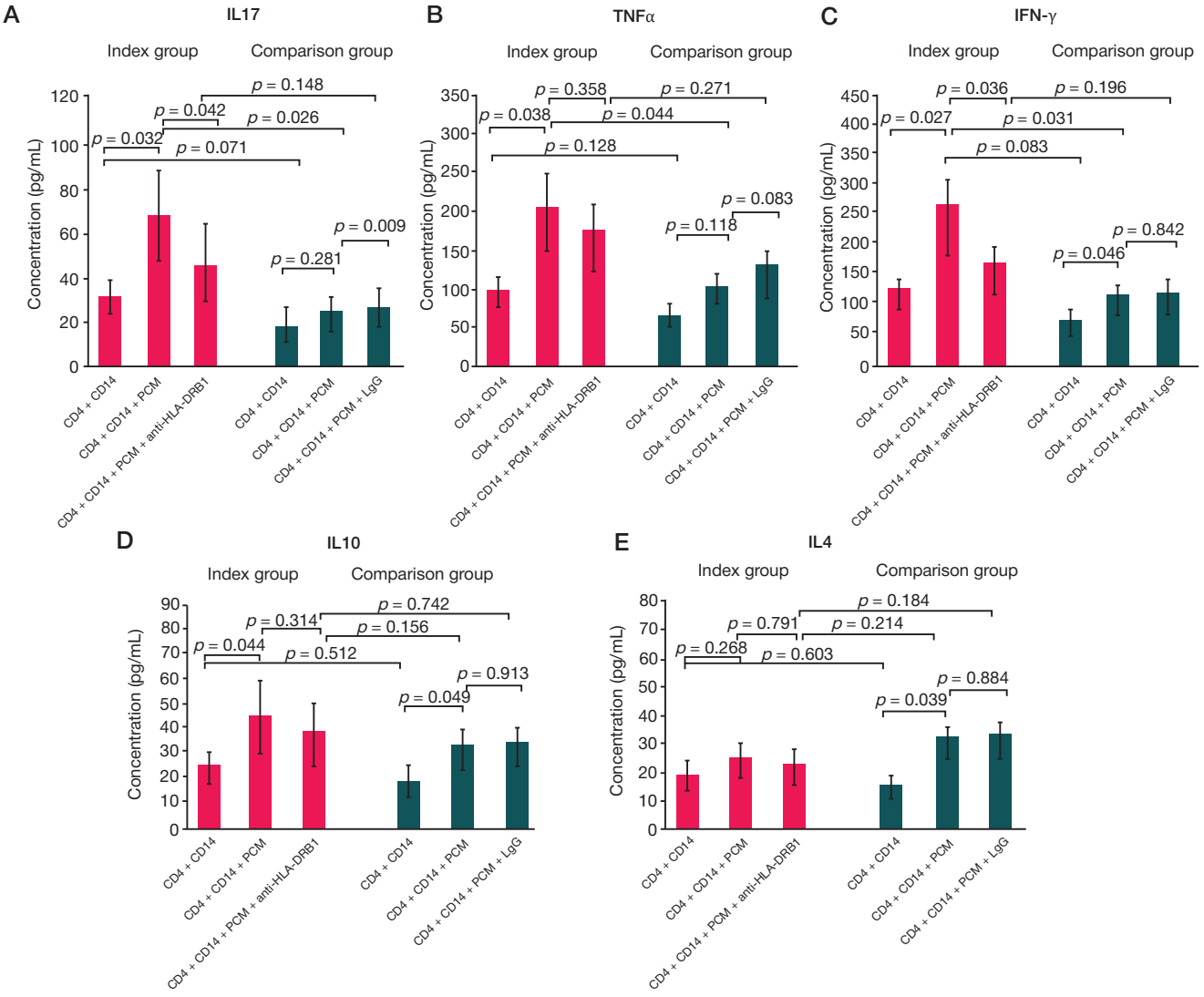

Fig. 3. Cytokine profile of supernatant under various co-culture conditions. IL17 **(A)**, TNFα **(B)**, IFN-γ **(C)**, IL10 **(D)**, and IL4 **(E)** levels in the index group and comparison group are provided. Data are presented as Me (Q_1–Q_3). The nonparametric Mann–Whitney U-test was used to compare independent groups; the Wilcoxon test for related samples was used for intragroup comparisons

an effect on the cytokine profile compared to the $CD4^+CD14^+$ PCM condition.

DISCUSSION

Today, numerous biomedical studies are focused on the role of HLA class II molecules in reproductive immunology. There are reports of many examples of the HLA-DRB1 locus allele encoding a certain amino acid sequence ("shared epitope") carrier state with reproductive and obstetric disorders, including PE [12–22]. Molecular mechanisms of these associations are still poorly uderstood. Modern ideas about the antigen presentation mechanisms make it possible to hypothesize the HLA-mediated nature of PE. Genetic variation of the HLA complex and the related receptor systems can affect the effectiveness of the fetal antigen complex recognition and presentation, which contributes to the T-cell immune response dysregulation. It should be noted that no potential target antigen associated with the development of PE, like many other disorders, has yet been identified. Furthermore, as discussed above, the MHC II-dependent antigen presentation might not be the only mechanistic basis of the HLA molecule association with the immune dysregulation in this pregnancy complication [24].

We conducted a series of experiments focused on assessing the influence of the HLA-DRB1*01:01 allele expressed on the surface of antigen-presenting cells on the Th1/Th2/Th17/Treg immunoregulatory axis imbalance in PE and NP in order to determine the contribution of maternal immunogenetic factors on the development of pregnancy complications.

Considering the key role of monocytes in regulation of pro-inflammatory and anti-inflammatory processes, in our study we focused on these cells. The $CD14^+$ monocytes obtained from patients with PE and NP were used in the study. The HLA-DRB1 molecules expressed on the surface of monocytes differed from each other by only three amino acid residues in the third hypervariable region (TAHR) of the DRβ chain.

During the co-culture of $CD14^+$ monocytes (showing expression of the SE 70-QKRAA-74 motif in the TAHR of the DRβ chain) and $CD4^+$ T cells obtained from patients with PE. The latter showed the trend toward the pro-inflammatory immune response development. At the same time, in similar experiments with the $CD14^+$ monocytes obtained from women of the comparison group (showing expression of the 70-DERAA-74 sequence), the $CD4^+$ T cells showed predominantly immunoregulatory potential. It should be taken into account that the study design features make it impossible to determine the priority of the HLA-DRB1*01:01 value for the

development of immune alterations in PE. In this regard, the effects observed can reflect both the HLA genotype effects and the immune regulation specifics resulting from PE. This circumstance determines the need for further research with the inclusion of additional comparison groups for more accurate data interpretation. A number of authors report the activated state of monocytes in patients with PE compared to monocytes obtained from women with NP [25, 26]. The findings of one experimental study show that the dendritic cells derived from peripheral blood monocytes of women with PE have the increased capacity for inducing the $CD4^+$ T cell differentiation in the direction of Th1 and Th17, including due to the enhanced expression of co-stimulatory molecules and increased IL23 secretion [27].

To explore the functional significance of the HLA-mediated presentation of placental antigens for the immune response development in pregnancy, in the next series of experiments monocytes were co-cultured with $CD4^+$ T cells in the presence of placental factors. It should be taken into account that in this study the PCM represents a complex biological substrate containing a broad spectrum of factors, including pro-inflammatory cytokines, anti-angiogenic molecules, extracellular vesicles and damage-associated molecular patterns (DAMPs), as well as specific fetoplacental proteins. It should be noted that it is precisely the placental metabolism disturbances of that are considered as a key component in the PE pathogenesis by a number of researchers. The central role of angiogenic factors, including soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), and soluble endoglin (sEng), the imbalance of which contributes to endothelial dysfunction and the systemic inflammatory response, has been proven [12]. The data on fetoplacental proteins, the impaired of production in PE can contribute to the immune dysregulation and the pro-inflammatory microenvironment formation at the maternal-fetal interface, deserve special attention. It has been found that the decreased production of human chorionic gonadotropin (hCG) is associated with the decreased counts of uterine and peripheral Treg cells and the impaired regulation of the uterine natural killer (uNK cell) population [13, 14]. The important immunoregulatory factors also include glycodelin A protein and α-fetoprotein. It has been shown that glycodelin A inhibits proliferation and cytotoxic activity of T cells and modulates the dendritic cell functions [15, 16], while α-fetoprotein can regulate the activity of lymphocytes and antigen-presenting cells, contributing to the immune homeostasis maintenance in the maternal-fetal system [17, 18].

In this regard, the observed PCM effects on the T-cell response are likely to result from the combined effects of various components capable of influencing both antigen-presenting cells and directly T cells. In the case of PE the immune cell co-culture in PCM further shifted the immune response towards the pro-inflammatory immune response, which was confirmed by the significant increase in TNFα, INF-γ, and IL17 cytokine concentrations. In contrast, the Treg cell counts and the immunoregulatory IL10 concentration significantly decreased compared with the similar experiment without PCM. According to current concepts, placental factors play a key role in the immune microenvironment regulation during gestation. In PE, under the conditions of placental ischemia, molecular signaling pathways are activated, initiating the massive release of damage-associated molecular fragments (DAMP), extracellular vesicles, and antiangiogenic factors, which, in turn, promote activation of maternal innate immunity components and the cascade enhancement of inflammation [2]. It has been shown that the syncytiotrophoblast membrane microparticles (STBM) isolated *in vitro* from the placenta of patients with PE induce activation of monocytes, increasing the CD54 expression and stimulation the pro-inflammatory IL6 and IL8 production. The reported data suggest a potential role of placental microparticles in the maternal systemic inflammatory response development in PE [28].

The co-culture of immune cells with PCM isolated from patients of the comparison group accomplished within the framework of this experimental study, on the contrary, was accompanied by the increase in Treg cell counts, elevation of the IL4 and IL10 levels amid the significant decrease in INF-γ levels compared to the similar experiment in the index group. The findings suggest the decrease in the Th1/Th2 and Th17/Treg ratios associated with the formation of the tolerogenic immune microenvironment playing a key role in maintaining the normal pregnancy course [29]. Considering significant intragroup differences in the experiments involving and not involving adding PCM, the important contribution of placental factors is clear. One study yielded the results that were comparable with our findings showing that exosomes isolated from the placenta in the 1st trimester of pregnancy (pEXO) induce the immune response "reprogramming" at the systemic level through modulation of the monocyte and T cell phenotype/function. According to the authors, pEXO contributed to induction of the M2-like polarization of macrophages with the increased expression of CD163, CD206, CD209, IL10, and IDO-1 and the reduced secretory potential with respect to the IFN-γ and TNFα pro-inflammatory cytokines. Furthermore, under the conditions of co-culture monocytes previously exposed to pEXO induced a two-fold increase in the $CD4^+CD25^+FoxP3^+$-Treg abundance, which suggests a functional role of the pEXO-mediated monocyte reprogramming in the T-cell response regulation and tolerogenic immune microenvironment maintenance in normal pregnancy. The PD-L1 expression increase on the monocyte surface can be a possible mechanism underlying this effect. However, further experimental studies are required to confirm the causal role of such pathway in the Treg induction [30].

In our experiment, the HLA-DRB1*01:01 blockage associated with PE was accompanied by the significant decrease in the IL17 and IFN-γ concentrations compared to the similar experiment not involving adding the blocking antibody. The data obtained suggest a possible role of maternal immunogenetic factors, including the carrier state for the HLA-DRB1*01:01 allele of the group of "shared epitope" alleles, in regulation of the immune interaction at the maternal-fetal interface. The detected decrease in the IL17 and IFN-γ levels can be considered as an indirect sign of changes in the antigen-presentating function of the innate immunity cells, including the MHC II/HLA-DR-dependent activation of $CD4^+$ T cells [31]. When interpreting the results obtained with the HLA-DR blockage, it should be taken into account that the monoclonal antibodies used target HLA-DR molecules in general and do not possesss allele specificity. In this regard, the observed cytokine profile alterations are likely to reflect the MHC II-dependent antigen presentation contribution to the T-cell response regulation. At the same time, the data obtained do not allow us to unambiguously attribute the effects identified exclusively to the influence of the HLA-DRB1*01:01 allele, so further research is required to clarify its specific role. In contast to cytokines associated primarily with the MHC II-dependent activation of $CD4^+$ T cells, the relatively preserved elevated TNFα levels can result from the direct activation of monocytes by placental factors, as well as additional FcγR-mediated stimulation of monocytes by immune complexes [32].

In the comparison group, adding anti-IgG to the culture medium did not lead to significant changes in the studied parameters compared to the conditions without adding the isotypic control. However, the reported upward trend of TNFα concentration suggests the possible nonspecific monocyte activation resulting from the interaction between Fc fragments of antibodies and Fcγ receptors on the monocyte surface.

Thus, the series of experiments conducted demonstrates potential importance of HLA-dependent antigen presentation mechanisms for the immune response regulation in PE and emphasizes the key role of placental factors in the immune microenvironment modulation at the maternal-fetal interface. At the same time, the study results should be interpreted carefully due to small sample size.

Study limitations

The study limitations result from the features of its design. The study was initially focused on assessing immunoregulatory effects of the HLA-DRB1*01:01 allele previously identified as a genetic factor associated with the increased risk of PE. At the same time, the lack of the group of patients with PE not being the HLA-DRB1*01:01 carriers makes it impossible to fully distinguish the influence of genetic predisposition and placental factors on the observed immune response alterations.

Moreover, the current study involved the use of a standard model of polyclonal T cell activation through the CD3/CD28 stimulation allowing us to assess the PCM effect on the functional response of the activated immunocompetent cells. However, the question regarding the potential for direct activation of T cells by the placental secretome constituents remains unresolved in this study. The above limitations determine the need for further research aimed to confirm the data obtained.

CONCLUSIONS

The study demonstrates the fundamentally new role of placental factors as active T-cell immune response modulators capable of determining the direction of the $CD4^+$ T cell polarization during gestation. It has been shown that in individuals with PE the placental conditioned medium induces a pronounced shift toward the pro-inflammatory phenotype accompanied by the decrease in Treg cell counts and the increase in pro-inflammatory cytokine production, while during normal pregnancy the same factors, on the contrary, shape the tolerogenic imme environment associated with the elevated immunoregulatory molecule levels. The contribution of HLA-DR-dependent mechanisms to the immune response regulation in PE associated with the HLA-DRB1*01:01 carrier state has been determined. It has been found that the HLA-DR-dependent interaction blockage is accompanied by the decrease in the IFN-γ and IL17 production, which suggests involvement of the MHC II-mediated $CD4^+$ T cell activation mechanisms in the pro-inflammatory immune response maintainance in PE. It can be assumed that variability of HLA class II molecules, including alleles of the “shared epitope” group, can contribute to the immune response regulation at the maternal-fetal interface and determine the features of the interplay between the placental microenvironment and the maternal immune system. The findings shape a new conceptual model, due to which PE can be considered as a condition, when the placental factors interacting with the immunogenetically determined characteristics of a pregnant woman can shift the immune homeostasis toward the pro-inflammatory phenotype. However, further research is necessary to confirm and expand the obtained data.

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