Research Article

Effect of Myelin Oligodendrocyte Glycoprotein (MOG$_{35-55}$) on Cell Differentiation and Antibodies-Abzymes Production in Transgenic EAE-Prone Th Mice with T Cells Response during the Development of Experimental Encephalomyelitis

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Abstract

The mechanisms of multiple sclerosis development are still unknown. It was shown that the development of Experimental Autoimmune Encephalomyelitis (EAE) in EAE prone C57BL/6mice (model mimicking human multiple sclerosis) having B and T lymphocyte responses is associated with modification in the differentiation profiles of bone marrow Hematopoietic Stem Cells (HSCs) and the increase in lymphocyte proliferation. T cell responses characterize other EAE transgenic prone Th mice. Different characteristics of the autoimmune reaction in Th mice were analyzed. During the development of EAE (and inflammation processes), the differentiation profiles of Th mice bone marrow HSCs (BFU-E, CFU-E, CFU-GM, CFU-GEMM, T and B lymphocytes) were noticeably or significantly different in male and female mice before and after their immunization with myelin oligodendrocyte glycoprotein (MOG$_{35-55}$).The patterns of B and T (including CD4 and CD8 cells) lymphocytes proliferation in several organs (spleen, thymus, bone marrow, blood and lymph nodes) during spontaneous (completely untreated mice) and MOG-treatment-accelerated development of EAE was also remarkably or significantly different in male and female mice. All these changes in male and female mice, despite some differences, were coupled with the increase in the concentrations of autoantibodies against DNA, myelin basic protein, and MOG, and with the increase in the relative activity of catalytic antibodies hydrolyzing these antigens. A comparison of the changes in a large number of parameters characterizing the development of EAE in Th and C57BL/6 mice was carried out. It was shown that MOG very much accelerates the development of EAE in Th mice. Despite some differences, the general patterns of the developing of spontaneous and MOG-accelerated EAE in Th male and female mice and in C57BL/6 mice are similar to a notable extent.

Keywords: Transgenic Th mice with T lymphocytes response; Experimental autoimmune encephalomyelitis development; Hematopoietic stem cells differentiation; Lymphocyte proliferation in different organs; Catalytic antibodies

Abbreviations

Abs: Antibodies; auto-Abs: Autoantibodies; AI: Autoimmune; AIDs: Autoimmune Diseases; BFU-E: Erythroid Burst-Forming Unit (early erythroid colonies); CFU-GM: Granulocyte-Macrophage Colony-Forming Unit; CFU-E: Erythroid Burst-Forming Unit (late erythroid colonies); CBA: (CBAxC57BL)F1 Mice; CFU-GEMM: Granulocyte-Erythroid-Megakaryocyte-Macrophage Colony-Forming Unit; CSF: Cerebrospinal Fluid; CNS: Central Nervous System; EPO: Erythropoietin; EAE: Experimental Autoimmune Encephalomyelitis; HSCs: Hematopoietic Stem Cells; IgG: Immunoglobulin G; IL: Interleukin; MBP: Myelin Basic Protein; MOG$_{35-55}$: Myelin Oligodendrocyte Glycoprotein; MTT: Tetrazolium Dye MTT 3-(4,5-methylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; MS: Multiple Sclerosis; SDS-PAGE: Sodiumdodecyl Sulfate-Polyacrylamide Gel Electrophoresis; sc; Supercoiled; scDNA: Supercoiled DNA; SLE: Systemic Lupus Erythematosus

Introduction

Multiple Sclerosis (MS) is the pathology of the Central Nervous System (CNS) related to an increase in T lymphocytes and macrophages. The precise route of multiple sclerosis is unknown [1]. Different studies support the essential role of the destruction of myelin due to Autoimmune (AI) reactions and inflammation processes. The activated myelin-active CD4+cells could be mediators of MS. Some findings also confirm the critical role of B cells and Autoantibodies (auto-Abs) to various myelin autoantigens in MS pathogenesis [1-3] The increased amounts of autoantibodies and the stockpiling of B cells in the bone marrow Cerebrospinal Fluid (CSF), as well as the MS patient's typical lesions, ensure key evidence for the demyelination involvement in the humoral response [4]. Studies of MS animal models indicate auto-Abs against myelin components are involved in Abs-dependent demyelination [3]. Autoantibodies against cell protein-oligodendrocyte progenitors could interfere with remyelination by

removing or obstructing these cells [5]. Autoimmune Diseases (AIDs) were first being proposed are originated from Hematopoietic Stem Cells (HSCs) defects [6]. Later, it was identified that the spontaneous (without mice immunization with any antigens), as well as antigen-induced development of EAE in C57BL/6 [7,8] and in MRL-lpr/lpr mice of Systemic Lupus Erythematosus (SLE) [9-11], is reached due to specific immune reorganization of bone marrow hematopoietic stem cells. The bone marrow immune system defects include specific parallel changes in the profile of differentiation of bone marrow HSCs and the production of catalytic

Autoantibodies-abzymes splitting DNA, RNA, polysaccharides, proteins, and peptides. The detection of Abs with catalytic activities is the statistically most significant and earliest marker of many AIDs in humans and animals [12-17] as well as in SLE [18-20], EAE [7,8] and MS [21-28]. Enzymatic activities of abzymes are well detected at the very initial stages of the diseases (at the pre-disease onset stage) before the emerging of typical markers of different AIDs [13-17]. Titers of auto-Abs to various auto-antigens at the onset of different AIDs usually correspond to typical indices' ranges corresponding to healthy humans and experimental mice. The appearance of multiple abzymes clear indicates the start of AIDs, while an increase in their enzymatic activities is associated with deep pathologies development. However, different AIDs development might be mediated byseveral mechanisms, eventually leading to a self-tolerance breakdown and inflammation processes. Several EAE models mimic particular aspects of human MS, including C57BL/6 mice (for a review, see [29-32]). In C57BL/6 mice, EAE passes as a spontaneous chronic- progressive disease. These mice show the specific T and B lymphocyte responses to antigens [29-32]. C57BL/6 mice were used to analyze eventual mechanisms of spontaneous DNA- and myelin oligodendrocyte glycoprotein (MOG35-55)-dependent acceleration of EAE development [7,8]. These mice's immunizations with MOG_{35-55} or DNA led to a speed-up of EAE development, associated with parallel specific changes in the profiles of differentiation of bone marrow HSCs, lymphocyte proliferation, and auto- Abs production possessing Myelin Basic Protein (MBP)-, MOG- and DNA-hydrolyzing activities. Another Th model corresponding to spontaneous EAE autoimmunity of CNS exists. This model was obtained due to crossing transgenic mice with a particular myelin-specific T-Cell Receptor (TCR) and mice specific for myelin-specific immunoglobulin heavy chain knock-in mice and described in [33]. Th mice demonstrate T cell responses to various antigens and primarily MOG, resulting in the spontaneous and stimulated development of a severe EAE. It is important to understand all possible complementary parallel mechanisms of disease to explain how MS and EAE develop. Here, we have carried out for the first time the analysis of many different parameters, including abzymes, characterizing accelerated development of EAE after Th mice immunization with MOG and compared the features of the development of pathology before and after the mice immunization. In addition, the same parameters were compared for Th mice with T-response and C57BL/6 with T and B-cell responses before and after mice immunization with MOG at different stages of EAE development.

Material and Methods

Chemicals

Protein G-Sepharose and Superdex 200 HR 10/30 columns were obtained from GE Healthcare (New York, USA), bovine polymeric DNA, and other different proteins and chemicals were from Sigma-Aldrich (Munich, Germany). 18.5kDa human MBP was from the Research Center of Molecular Diagnostics and Therapy (RCMDT, Moscow, Russia) and mouse oligopeptide MOG_{35-55} was perched from EZBiolab (Germany). All preparations were free from any possible contaminants. Methylcellulose-based M3434 medium was from StemCell Technologies (Canada) and RBC lysis buffer from Biolegend (San Diego, CA, USA). Fetal bovine serum was from Invitrogen (Waltham, MA, USA)

Experimental animals and ethical statement

Th mice line with T-cell response was matured in Germany (Westfälische Wilhelms- Universität, Department of Neurology, Münster). The main characteristics of this line of mice, they are predisposed to the development of EAE, are described in [33]. Inbred Th mice (3 months of age at the beginning of all experiments) were grown in the Institute of Cytology and Genetics (ICG) special mouse breeding facility in standard conditions free of any pathogens. All experiments were carried out with mice pursuant to protocols of the Bioethical Committee of the Institute of Cytology and Genetics (document number 134A of 07 September 2010), satisfying the humane principles of the European Communities Council Directive (86/609/CEE) for working with animals. The Bioethical Committee of ICG supported this study. The relative overtime weight of male and female mice and degree of proteinuria (concentration of total protein in the urine, mg/ml) were analyzed as in [9-11]. For comparison, we used C57BL/6 wild-type mice described earlier [7,8,31].

Immunization of mice

Analysis of changes in various parameters, characterizing the development of EAE during 70-83 days, was carried out in the case of groups of seven female and male mice without any treatment and after their immunization with MOG. Immunization (ortreatment) of Th mice with MOG_{35-55} was performed using MOG_{35-55} and Pertussis toxin according to a previously published protocol [31] used previously in [7]. On day 1 (zero time of experiments), Th mice were treated by injection of 30μg of MOG35-55 per mouse in the back, two times in the left and right side using 20μl of Freund's complete adjuvant containing Pertussis toxin (400ng/mouse; Mycobacterium tuberculosis). The next day 20μl of Pertussis Toxin (400ng/mouse) was additionally injected in a similar way. For different experiments, 0.7-1ml of the blood was collected after decapitation using standard approaches.

ELISA of anti-proteins and anti-DNA antibodies

Anti-DNA Abs analysis (the sera were diluted 100-fold) was performed using the ELISA test system of ORGENTEC Diagnostika (Germany) according to the manufacturer's instructions as in [7-9,34,35]. The relative contents of IgGs against MBP and MOG35-55 were estimated using purified polyclonal electro phoretically homogeneous Abs as in [7-9,34,35]. After all previously described stages of the treatment of immobilized MBP and MOG and bound with them IgGs, rabbit anti-mouse Abs conjugated with horseradish peroxidase were added; all samples were incubated with H_2O_2 and then with tetramethylbenzidine. The optical densities of the solutions (A_{450}; after adding H_2SO_4) were measured using the Uniskan II plate reader (MTX Lab Systems, USA) [7-9]. The relative A450 values

of the samples were calculated from differences in A_{450} between experimental and control solutions containing no DNA, MBP, or MOG. More detailed data concerning the determination of anti-DNA and anti-proteins concentrations are given in Supplementary data (Part 1: "ELISA of anti-proteins and anti-DNA antibodies").

IgG purification

Electrophoretically homogeneous IgGs from blood sera of Th mice were obtained using affinity chromatography of sera components on Protein G-Sepharose and following FPLC gel filtration of IgGs in drastic conditions (pH2.6), destroying immunocomplexes as in [7,8,21-23,34,35]. More detailed data concerning these methods are given in Supplementary data (Part 2: "IgG purification"). IgGs were protected from viral and bacterial contamination by their filtration using Millex membranes (0.1μm). Analysis of IgGs by SDS-PAGE was carried out using gradient gels (4-15%) and visualized using silver staining according to [7,8,21-23,34,35].

DNA-hydrolyzing activity assay

DNase activity of IgGs was estimated according to [9,24,25]. The mixtures (20μL) containing 20mM Tris-HCl (pH 7.5) supplemented with 20mM NaCl, 1mM Ethylenediaminetetraacetic Acid (EDTA), 5mM $MgCl_2$, 20μg/ml Supercoiled (sc) pBluescript and 0.001-0.1 mg/ml of IgGs. The samples were incubated during 5-24 h at 37°C. The products of DNA splitting were analyzed using 0.8% agarose gels electrophoresis. Photographs of ethidium bromide-colored gels were analyzed by Gel-Pro Analyzer v9.11 (Media Cybernetics, L.P., Germany). The Relative Catalytic Activities (RAs) were calculated from the difference between the relative amount of intact Supercoiled DNA (scDNA) and its hydrolyzed-relaxed form, taking into account DNA-substrate distribution between these two bands after scDNA incubation without IgGs. All initial DNA splitting rates were analyzed from linear parts of the reaction time dependencies (30-40% of scDNA hydrolysis) and concentrations of Abs providing 30-40% of scDNA hydrolysis. A complete transition of scDNA to its relaxed form was taken for 100% of the activity. The relative activities (% of the hydrolysis) were finally recalculated to the same standard conditions; time and IgGs concentration (mole of sc DNA /min/mg of IgGs)

Protease activity assay

The mixtures (10-50μL) containing 20mM Tris-HCl buffer (pH7.5) supplemented with 0.7-1.0mg/ml of proteins (MBP or MOG), as well as 0.001-0.2 mg/mL of IgGs were incubated for 5-24 h at 37°C [7,8,21-23,34,35]. The proteins cleavage products were estimated by SDS-PAGE using 12% or 3-15% gradient gels with the following staining Coomassie R250. The gels were scanned, and hydrolyzed products were quantified by GelPro v3.1 software. IgG samples' relative activities were evaluated from a decrease (%) in the initial MOG or MBP transited to their hydrolyzed forms. The hydrolysis of the proteins incubated without antibodies was taken into account. All initial hydrolysis rates were calculated using pseudo-first-order reaction conditions, linear regions of reactions time dependencies, and IgGs concentrations (20-40% hydrolysis of the proteins). The relative activities (% of the hydrolysis) were finally recalculated to the same standard conditions; time and IgGs concentration (mole of protein (MBP or MOG) / min/mg of IgGs).

SDS-PAGE analysis of catalytic activities

To prove that antibody activity is their own property, the analysis of DNA- and different proteins-hydrolyzing activities of Th mice IgGs after SDS-PAGE was performed as in [7,8,21-23,34,35]. These methods have been developed and published previously and are given in Supplementary data (Part 3: "SDS-PAGE analysis of catalytic activities").

Analysis of bone marrow progenitor cells in culture

Samples of bone marrow were obtained from 14 mouse femurs, and then the ability of bone marrow cells to form different colonies was estimated as in [7,8,34,35]. The standard methylcellulose-based M3434 medium specific for mouse cells was used; four dishes per mouse (2×10^4 cells) were grown. The medium contained Erythropoietin (EPO), stem cell factor and interleukins IL-3 and IL-6. The relative number of CFU-GM (Granulocyte-Macrophage Colony-Forming Unit), CFU-GEMM (Granulocyte-Erythroid-Megakaryocyte- Macrophage Colony-Forming Unit), BFU-E (Erythroid Burst-Forming Unit, Early Erythroid Forming Unit) and CFU-E (Erythroid Burst-Forming Unit, Late Erythroid Forming Unit) cell colonies on the dishes was calculated after 14 days of incubation at 37°C (5% CO_2) in a humidified incubator as in [7,8,34,35].

Evaluation of lymphocytes in different mouse tissue samples

The relative content of B and T lymphocytes in various organs and blood of mice was estimated by flow cytometry. Peripheral blood was obtained using mice's standard decapitation. Sodium citrate was used as an anticoagulant. Five hundred thousand leukocytes in not more than 150μL were used for cytometry analysis. Cells were incubated with monoclonal Abs for 20min in the darkness, then the cells of blood samples were lysed using a special 10-fold volume of RBC lysis buffer for 20min, centrifuged for 10min, and washed using 500μL of PBS buffer (150mM NaCl, 17mM KH_2PO_4, 52mM Na_2HPO_4) containing 0.02% EDTA and 1% sodium azide. After samples centrifugation, 50μL of PBS buffer was added to the cell pellet, and they were analyzed by a flow cytometer. Lymphocytes were isolated from blood, bone marrow, thymus, lymph nodes, and spleen. Bone marrow was derived by rinsing the femoral cavity. Lymph nodes and thymus samples were carefully homogenized, large particles were removed and different cells were resuspended by their passing using a disposable syringe through a needle. Spleen cells were received by washing this organ with a medium-filled syringe through punctures in the spleen stroma. This approach allows the obtaining of splenocytes without impurities from the stroma of the spleen. Cells were then washed by their centrifugation with RPMI- 1640 medium (5ml) at 1500rpm for 10min. After the second centrifugation, RPMI-1640 medium (1ml) containing 10mM HEPES, 10% fetal bovine serum, 0.5mM 2- mercaptoethanol, 2mM L-glutamine, 100μg/ml benzylpenicillin, and 80μg/ml gentamicin were added to the cell pellets, and the cells were counted. The relative cell content in extracts of various organs was analyzed using 500 thousand cells in 100μL of PBS buffer supplemented with 10% fetal bovine serum and the conjugates of different specific monoclonal antibodies. To analyze the relative amount of different cells, specific antiCD45-BV510 (Biolegendcat # 103138), antiCD3-FITC (Biolegendcat # 100204), antiCD4-PerCP (Biolegendcat # 100432), antiCD8alpha-APC

(Biolegendcat # 126614), and antiCD19-PE (Biolegendcat # 115508) antibodies were used. All staining of cells was carried out according to the manufacturer's recommendations. Cells were incubated for 20 min with monoclonal antibodies, washed by centrifugation after adding 500μL of PBS. 50μL of PBS after centrifugation was added to the cell pellet, and the mixtures were used for analysis using the BD FacsVerse flow cytometer (BD Biosciences, SanJose, CA, USA). More than 100,000 events were collected for each sample. Gating was performed as follows: the total cell population was isolated according to cell size and granularity, and the white blood cell population was determined using the pan-white blood cell marker CD45+ and populations of CD3+ and CD3⁻leukocytes were obtained. In the CD3+ leukocytes population (T cells), CD4+ and CD8+T cells were determined, and in the CD3– leukocytes population, the relative content of CD19+ B cells was found. For all groups, the percentage ratio was calculated relative to the initial lymphocyte population.

Statistical analysis

The final determined values are given as the mean±S.D. of three independent experiments for each mouse (7 male and 7 female mice); the data were averaged over seven different male and female mice of each group. Some of the sets of samples did not match the Gaussian distribution. Therefore, to estimate the differences between parameters analyzed, the Mann-Whitney U test was utilized; $P < 0.05$ was regarded statistically significant.

Results

Choosing a model for studying EAE development

T and B lymphocytes play vitally important roles in the pathogenesis of human MS [1] and animals EAE [29-32]. B cells provide Abs important for the humoral immunity of the adaptive immune system [32]. In the bone marrow, mature B cells have membrane receptors allowing them to interact with different antigens leading to the initiation of the Abs response. Spontaneous and MOG-accelerated EAE in C57BL/6 mice are characterized by both T and B cell responses [29-32]. C57BL/6 mice spontaneous development of EAE associates with slow changes in HSCs differentiation profiles parallel with an increase in the level of lymphocyte proliferation in various organs during the initial 2-3 months [7,8,34,35]. These mice's changes lead to the parallel production of abzymes hydrolyzing MOG, MBP and DNA [7,8,34,35]. Immunizing C57BL/6 mice with MOG accounts for a powerful acceleration of EAE development

with the onset and acute phases appearing at 7 and 14-20 days after immunization, respectively [7,8,29-32,34,35]. During these phases, there was observed an increase in concentrations of auto-Abs and the appearance of abzymes hydrolyzing DNA, MBP and MOG. Such abzymes are very dangerous for mammals. Abzymes with DNase activity can penetrate through cellular and nuclear membranes, cleavage DNA of chromatin, and induced cell apoptosis [36-38]. This process results in an increase in blood concentration of DNA complexes with histones, which are the most important antigens in the production of Abs against DNA and histones [39]. Abzymes splitting MBP and MOG hydrolyze these components of nerve tissue membranes, leading to an impaired nerve impulse [13-19,21-23]. In contrast to EAE prone C57BL/6 mice having T and B cell responses [29-32], transgenic EAE prone Th mice are characterized by a T cell response [33]. Therefore, it was interesting to compare various parameters characterizing the spontaneous and MOG- accelerated development of EAE in Th and C57BL/6 mice. In the case of C57BL/6 mice, there was analyzed the development of EAE using only male mice [7, 8,34,35]. However, multiple sclerosis is less common for men than for women [40]. Only about a third of patients with MS are men, and the rest are women. In addition, the disease often occurs quite late in men and proceeds more severely. Therefore, taking this into account, in this study, we compared the development of EAE after immunization with MOG of male and female Th mice. In addition, to compare specific features of the EAE development in mice with T (Th) response and T and B (C57BL/6) responses, some previously obtained data on the analysis of C57BL/6 male mice were used [7,8,34,35].

Weight and proteinuria of EAE mice

We have first analyzed over time changes in the relative weight of Th mice before and after they immunization with MOG (Figure 1A). Interestingly, the immunization of male and female mice with MOG had minimal effect on their weight over time compared with the untreated mice. After the immunization, a decrease in weight of C57BL/6 males during 10-15 days (Supplementary S1; all supplementary Figures are given in Supplementary data) was more pronounced than in Th males (Figure 1A). Development of autoimmune pathologies in different autoimmune prone mammals, including MRL-lpr/lpr [9-11] and C57BL/6 mice [7,8,34,35], is characterized by proteinuria (3mg/ml concentration of protein in urine). Non-autoimmune CBA and BALB mice at least during
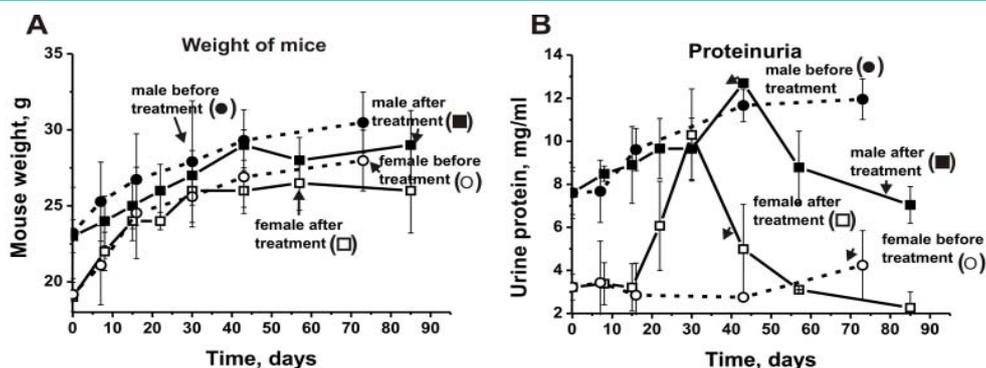

Figure 1: Overtime changes in weight (A) and proteinuria-relative concentration proteins in the urine of Th mice (B) before and after mice treatment-immunization with MOG.
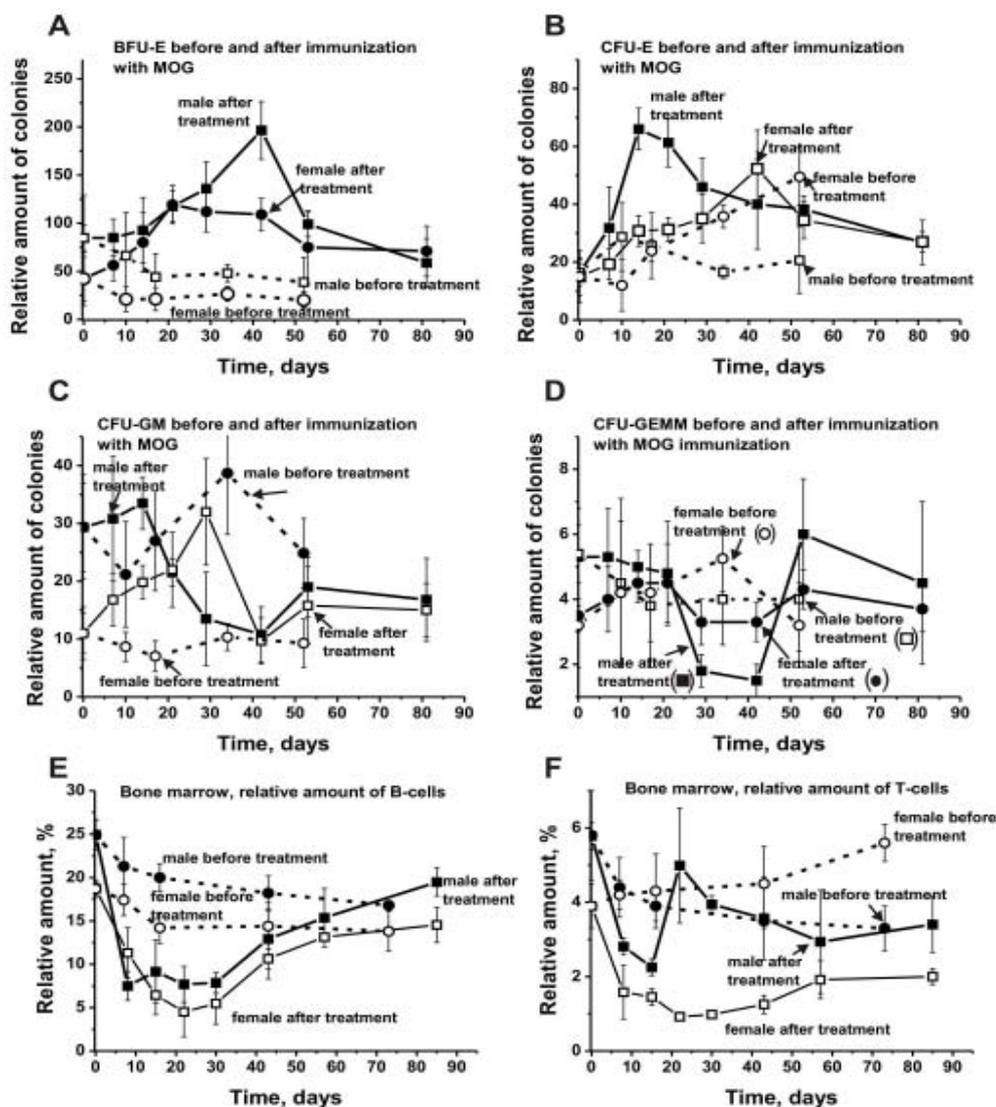
Figure 2: Overtime changes of the average content of bone marrow progenitor colony-forming units for untreated and MOG-treated mice (7 mice of each group): BFU-E (A), CFU-E (B), CFU-GM (C) and CFU-GEMM (D) as well as bone marrow B (E) and T (F) cells.

12months demonstrated the absence of proteinuria (0.1-0.12 mg/ml) [9-11]. Autoimmune-prone healthy MRL-lpr/lpr mice before the development of SLE are characterized by low proteinuria (0.38mg/ml) [9-11]. However, C57BL/6 male mice have demonstrated a higher level of protein concentration in urine (up to 10-12 mg/ml) [7,8,34,35]. The overtime changes in proteinuria of Th mice before and after their immunization with MOG were analyzed (Figure 1B). At time zero (3months of age) in two groups of seven male and female mice before their immunization, the average urine protein concentration was surprisingly very different: 7.6 ± 2.2 and 3.2 ± 1.0 mg/ml (P < 0.05), respectively (Figure 1B). During the spontaneous development of EAE, the concentration of proteins in the urine of Th males increased 1.6 times (to 12mg/ml), while in females, only 1.3 times (to 4.2mg/ml); the difference in concentration is statistically significant (p=0.01). After immunization of male mice, proteinuria was increased up to day 43, similarto its growth before mice treatment and then it began to decreased (Figure 1B). The difference is statistically significant

(P=0.01). In contrast to Th male mice, the immunization of C57BL/6 male mice led to a relatively flowing 1.8-fold increase in proteinuria after immunization compared with the spontaneous development of EAE (Supplementary Figure S1). A completely different situation was observed in Th female mice having at zero time lower proteinuria. In female mice from 15 to 30 days, proteinuria was sharply increased 3.2-fold (to 10.3 mg/ml) and then was decreased (Figure 1B). Thus, the immunization with MOG differently affects the proteinuria of female and male mice, which might be possibly associated with some specific features of EAE development in Th female and male mice.

Hematopoietic progenitor colony formation

It was shown that the differentiation profile of bone marrow stem cells in CBA and BALB mice not predisposed to AIDs does not remarkably change for at least 1-2 years [9-11]. It was shown that spontaneous in-time development of EAE in C57BL/6 mice (change in the analyzed parameters of EAE in time before immunization of
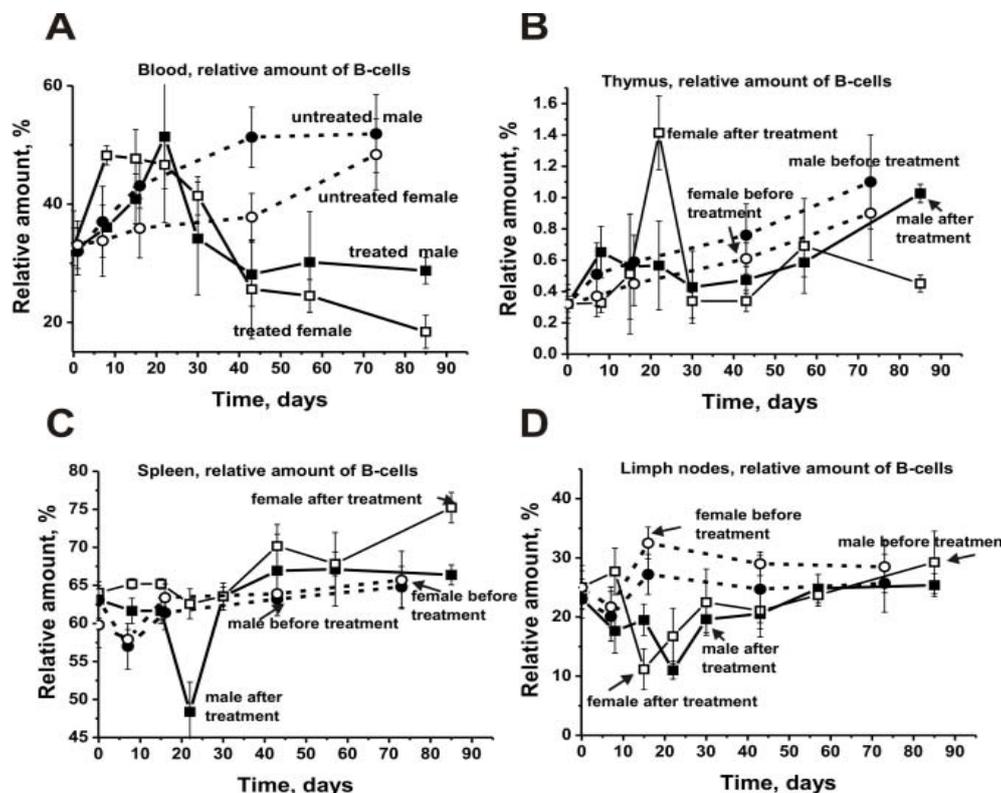
Figure 3: Overtime changes in B lymphocyte proliferation (%) in the blood (A), thymus (B), spleen (C), and lymph nodes (D) for untreated and MOG-treated mice. The error in B lymphocyte determination from three independent experiments in the case of each mouse of every group (7mice) did not exceed 7-10%.

mice) results in significant changes in the differentiation profile of bone marrow stem cells [7,8,34,35]. Therefore, we first performed carried out a parallel analysis of possible changes in the stem cell differentiation profiles of three-month-old EAE prone Th mice (7males and 7females; the maximum number of mice, which it was possible to analyze in such parallel experiments). During the 80 days of MOG-induced EAE development, a significant increase in the relative number of BFU-E colonies occurred in the bone marrow of Th male and female mice in the period from 15 to 40 days (Figure 2A). In the beginning, there was 2.0-fold difference ($P < 0.05$) between BFU-E colonies of male and female mice, but during the entire analysis period of EAE development did not differ significantly ($P = 0.19$) except 1.8-fold difference ($P < 0.05$) at day 42. Simultaneously, during thespontaneous development of EAE, the relative number of these colonies gradually decreases (Figure 2A), demonstrating a significant difference for male and female mice ($P = 0.02$). Earlier, we analyzed the changes in the differentiation profile of HSCs in C57BL/6 male mice with T- and B-responses. Interestingly, in male mice of C57BL/6 line, at the spontaneous development of EAE, there are no significant changes in the number of BFU-E colonies up to 20 days, while after immunization, it noticeably decreases (Supplementary Figure S2). After 20 days, the relative number of these colonies increases in both immunized and untreated C57BL/6 male mice. Th male mice before immunization do not demonstrate an increase, but a decrease in the number of these colonies over time, and after immunization, the increase in the number of BFU-E colonies begins earlier, from about 10-15 days of experiments (Figure 2A). A significant difference in the direction of changes in CFU-E colonies was observed between

female and male Th mice during spontaneous EAE development (Figure 2B). The number of CFU-E colonies in male mice increases during 10 days and then slowly decreases; only slight changes in the relative number of CFU-E colonies in Th female mice are observed up to approximately day 35 and then their number remarkably increases. There was no, however, the statistical difference in these average values during the development of EAE in male and female mice ($P > 0.05$). Immunization with MOG leads to a significant increase in CFU-E colonies in Th males and females with earlier and stronger growth of these colonies number in male mice (Figure 2B). A statistically significant ~2-fold difference ($P < 0.05$) in the number of colonies in males and females after their immunization with MOG was observed in the period from 14 to 21 days (onset and acute phase of EAE). Despite the significant difference in the profiles of changes in the relative average number of CFU-E colonies during the entire experiment, the difference in the colonies for female and male mice was insignificant, $P > 0.05$.C57BL/6 males showed a low increase in the number of these colonies (Supplementary Figure S2). nevertheless, in the case of males of both Th and C57BL/6 lines, their immunization with MOG results in a sharp increase in the number of these colonies by days 10-20 (Figures 2B and Supplementary Figure S2).The change in the average number of CFU-GM colonies over time during spontaneous development of EAE in female and male Th mice is complex (Figure 2C) and characterized by a significant difference ($P = 0.012$). There is a decrease in the CFU-GM colonies at 10-20 days, but a notable increase by day 35 and a further perceptible decrease. Th males showed an opposite change in the number of CFU-GM colonies before and after their immunization with MOG demonstrating a
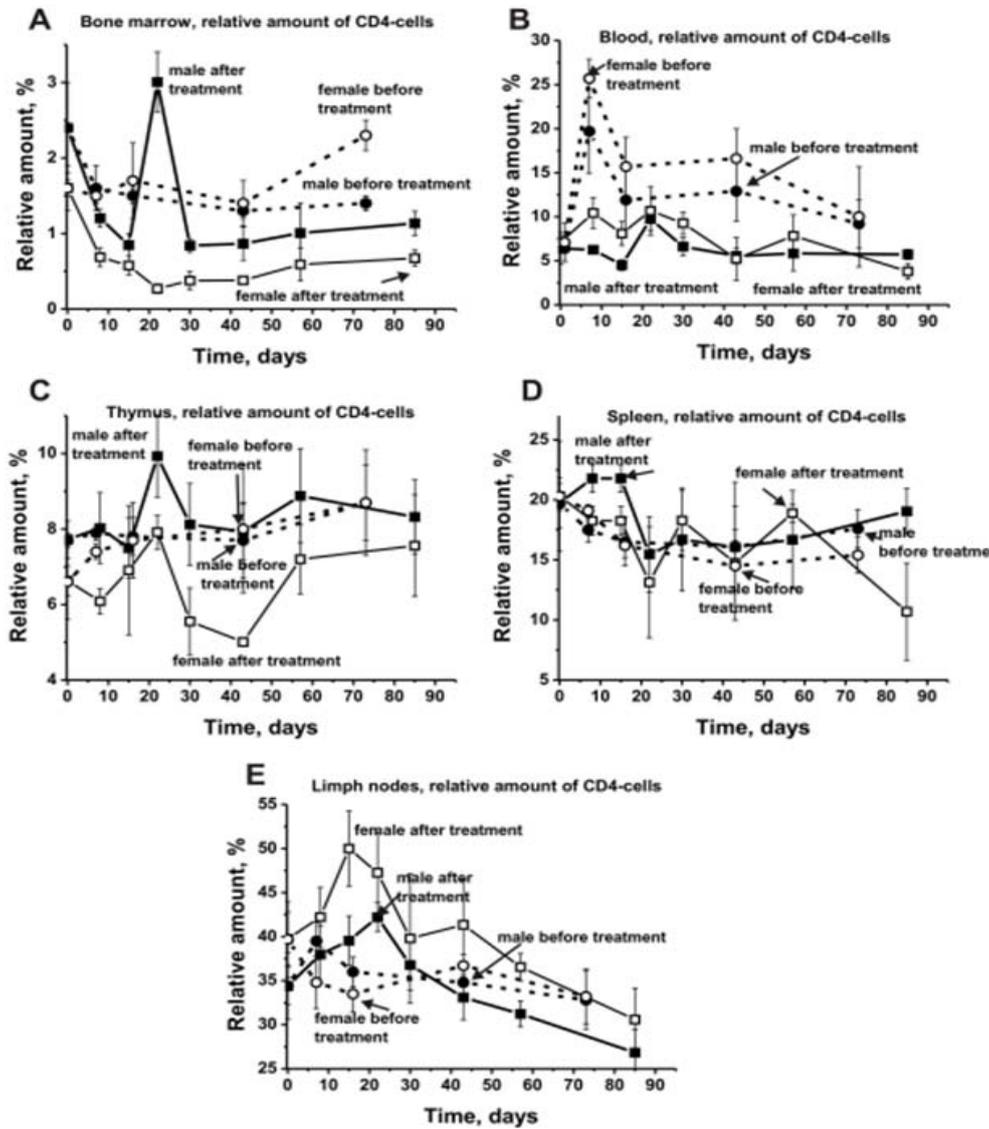
Figure 4: Overtime changes in CD4 lymphocyte proliferation (%) in bone marrow (A), blood (B), thymus (C), spleen (D), and lymph nodes (E) for untreated and MOG-treated mice. The error in CD4 lymphocyte determination from three independent experiments in the case of each mouse of every group (7mice) did not exceed 7-10%.

maximal 2.7-3.0-fold (P < 0.05) difference from 30 to 40 days. While before treatment, the number of colonies sharply decreased by 30-40 days after immunization of male mice, their number decreased significantly from 10 and 50 days (Figure 2C). No very significant changes were observed before immunization of Th female mice, but after their immunization with MOG, a sharp increase in their number occurred by 30 days (P = 0.015). In other words, there is an oppositely directed change in the CFU-GM colonies in Th males and females after their immunization - in males, a sharp decrease, and in females, a powerful increase in the number of colonies. In contrast to a sharp increase before and a sharp decrease of CFU-GM colonies after Th males immunization with MOG (Figure 2C), C57BL/6 males show a slow a gradual in time increase in their number during spontaneous and a slight decrease at day 10 during the MOG-induced development of EAE (Supplementary Figure S2). For CFU-GEMM colonies of Th mice during spontaneous development of EAE, there were utterly

opposite changes in the number of CFU-GEMM colonies for Th female and male mice: their number decreases in males but increases in female mice (Figure 2D). At the same time, treatment of Th females and males with MOG led to a sharp 2.0-2.8-fold (P < 0.05) decrease in the numbers of CFU-GEMM colonies for males at 30-40 days comparing with female mice (Figure 2D). In C57BL/6 males, there were gradual in time changes in CFU-GEMM colonies before and a faster decrease after their immunization (Supplementary Figure S2). The total amount of T and B cells in the bone marrow of Th female and male mice at the beginning of the experiment is significantly different (Figures 2E and 2F). Approximately the same 1.4-1.5-fold in time decrease in the number of B cells one can see for Th male and female mice (P > 0.05). There were observed overtime different character changes in the number of T cells in female and male mice bone marrow before immunization (Figure 2F). The relative number of T cells decreases in males but increases in female mice (P > 0.05).

After Th mice immunization with MOG, the number of B cells was significantly and comparable decreases in male and female mice from 7 to 30 days, and then it begins to increase, P > 0.05 (Figure 2E). After Th male and female mice immunization with MOG, a relative number of T cells was significantly decreases today 15 (Figure 2F). Later the number of T cells in the bone marrow of female mice continued to decrease smoothly. At the same time, in Th male mice, there was a sharp increase in the number of T cells with a maximum at day 23 (Figure 2F). Overall, there was a statistically significant difference in T cells of bone marrow in male and female mice after their immunization (P = 0.005). Thus, the changes observed in Th male and female mice during spontaneous development of EAE are different for CFU-E, CFU-GEMM, and T cells but, to some extent, similar for BFU-E, CFU-GM, and B cells (Figure 2). After immunization with MOG, some similarity in the change in the number of colonies is observed for BFU-E, CFU-E, CFU-GEMM, and B-cells but is opposite for CFU-GM colonies and T cells (Figure 2).

The content of B and cells in various organs of mice

Initially, we estimated, over time, relative average values characterizing the content of B and T lymphocytes in bone marrow (Figure 2) and then in other organs of Th male and female mice before immunization and after their treatment with MOG. The changes in relative amounts of B lymphocytes in different organs of mice are shown in (Figure 3). The number of B cells over time in the blood of male mice at spontaneous development of EAE grows smoothly and significantly faster for male than for female mice (Figure 3A). The maximum difference of 1.4 times (P < 0.05) is observed at 43 days after the start of the experiment. Immunization of Th mice with MOG leads to an approximately comparable strong increase in the number of B lymphocytes in the blood of males and female mice from 7 to 30 days, followed by a decrease in their number (Figure 3A). At 7 days after immunization, the relative number of B-cells in female is 1.3 times higher (P < 0.05) than in male mice. The number of B lymphocytes growth in the thymus of female and male mice proceeds smoothly and almost similar (Figure 3B). The curves of changes in the number of B lymphocytes in Th female and male mice after their immunization with MOG are very complex. At first, from 7 to 20 days, an increase in the number of B cells is observed (which is noticeably stronger in female mice) than a decrease (30-40 days) and again their growth (Figure 3B). A very keen increase in the number of B-lymphocytes (P < 0.05) was observed in female mice at 22 days. The complex patterns of changes in the number of B lymphocytes in the spleen and lymph nodes of Th male and female mice were slightly different. During the first 7-16 days, there was in both male and female mice a decrease in the number of B cells in the spleen, but an increase in lymph nodes, followed by a slight rise in spleen but a decrease in lymph nodes (Figures 3C and 3D). Mice immunization with MOG dramatically changed these patterns. In male mice spleens, there was a sharp decrease in B lymphocytes by day 22 (P < 0.05), and then there was a remarkable increase in their relative content (Figure 3C). In the spleen of female mice, there was a constant but salutatory increase in B lymphocytes. The patterns of reduction in B cells in the lymph nodes of male and female mice from 7 to 30 days after immunization mice with MOG were, to some extent, similar (Figure 3D). After immunization, there was a strong decrease of 2.3-2.4 times (P < 0.05) in the relative number of B-lymphocytes in 15 days in female and 22 days in male mice (Figure 3D). Taken together, the curves of the changes in the average relative concentration of B cells in various organs before and after immunization were different, but the characters of these changes in every organ of Th male and female mice were, to some extent similar (Figures 2 and 3).

The content of T cells in different organs of mice

The patterns of changes in the number of T lymphocytes in the bone marrow of Th male and female mice before and after the immunization were directly opposite. We evaluated overtime dependencies in the changes average relative number CD4 and CD8 lymphocytes in various organs before and after mice immunization with MOG. Th male mice before immunization during spontaneous EAE demonstrated during 20 days a slight decrease in the relative number of total CD4 cells in the bone marrow with access to the plateau after 20 days (Figure 4A). After immunization, a stronger decrease in the relative number of CD4 was observed with its very sharp increase on day 22 (P < 0.05). During the spontaneous development of EAE in Th female mice, the relative number of CD4 cells began to increase only after 40-50 days compared to zero time (Figure 4A). After immunization of Th female mice, a smooth but finally very strong decrease in average CD4 lymphocytes in the bone marrow (P = 0.015) in comparison with spontaneous EAE was observed (Figure 4A). During the spontaneous development of EAE, male and female mice demonstrated similar patterns of changes in CD4 cells in the blood with a sharp increase at 7-15 days after the start of the experiment. Immunization of Th male and female mice resulted in a strong decrease in the relative number of CD4 lymphocytes in their blood and complex jump-like patterns of changes in these cells over time (Figure 4B). The difference in the number of CD4 cells before and after immunization in males was statistically significant (P = 0.02) but not in females (P = 0.09). The maximum 4.1-fold difference (P < 0.05) in this parameter in females was observed at 8 days. The patterns of a slow increase in the average relative number of CD4 cells in the thymus of Th female and male mice during spontaneous development of EAE were similar (Figure 4C). The type of dependencies of CD4 cells changes in the thymus of Th female and male mice were also to some extent resembling a sharp increase at day 23 and the subsequent powerful decrease in the number of lymphocytes at day 40. However, changes in CD4 cells for male and female mice over time were nevertheless very different (P =0.003). Before immunization of Th male and female mice with MOG, they showed a similar slight overtime decrease in the amount of spleen CD4 lymphocytes (Figure 4D). The immunization of male mice with MOG led to a slight increase in the number of lymphocytes at 7-15 days with their subsequent decrease (Figure 4D). In female mice, after immunization with MOG, a very complex change in the number of CD4 lymphocytes was observed with a few weak highs and lows. More unambiguous and similar patterns of changes in the number of CD4 lymphocytes were found in the lymph nodes of Th male and female mice (Figure 4E). Prior to the immunization, the curves of overtime decrease in the number of CD4 cells in lymph nodes in female and male mice were comparable. After immunization of Th mice, there was a sharp increase in the number of CD4 cells in these organs of male and female mice from 7 to 23 days, and then there was a very strong decrease in their relative number (Figure 4E). The maximum 1.3-fold (P < 0.05) difference in the number of CD4 cells in
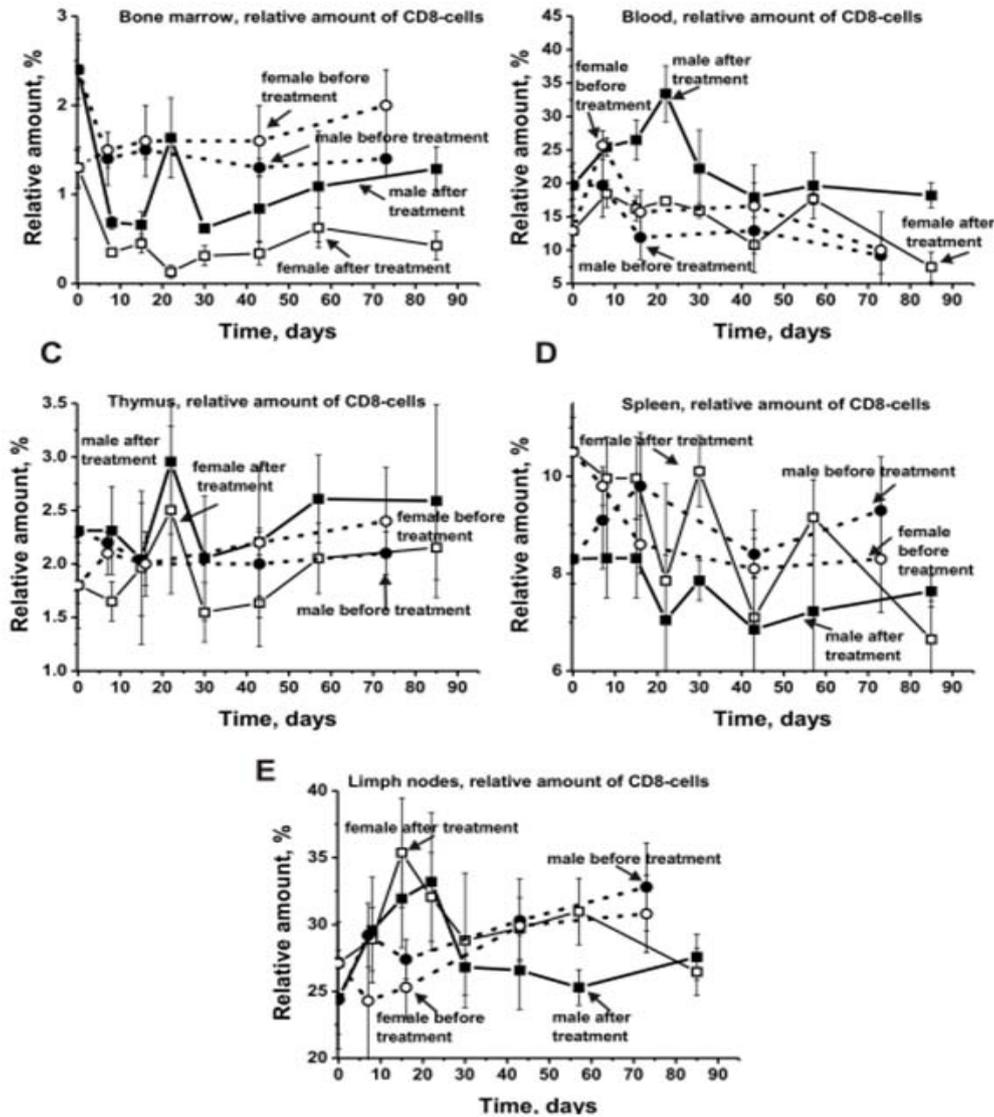
Figure 5: Overtime changes in CD8 lymphocyte proliferation (%) in bone marrow (A), blood (B), thymus (C), spleen (D), and lymph nodes (E) for untreated and MOG-treated mice. The error in CD4 lymphocyte determination from three independent experiments in the case of each mouse of every group (7mice) did not exceed 7-10%.

female and male mice after immunization was observed at 15 days. During the spontaneous development of EAE, the patterns of changes in CD8 cells in the bone marrow of Th mice are similar (Figure 5A) to overtime changes in CD4 lymphocytes (Figure 4A). In the first 7-15 days, there is a noticeable decrease in their average relative number for male but an increase for female mice (Figure 5A). Then the Th male mice demonstrated a plateau, while the female mice show an increase in CD8 cells after 40 days (Figure 5A). Interestingly, the patterns of overtime changes in the number of CD8 lymphocytes in the bone marrow of Th male and female mice (Figure 5A) also have a similar character to those for CD4 cells (Figure 4A). The number of CD8 cells in female mice sharply decreases by day 20 and reaches a plateau; the strong difference in average data before and after mice immunization during complete experiments is observed (P = 0.003). In the case of male mice, there is a strong decrease in the number of these lymphocytes following a sharp splash in their number on day 22

(Figure 5A). At 22 days, the difference in the number of cells of immunized female and male mice is 12.5 times (P = 0.007). The curves characterizing changes in CD8 and CD4 lymphocytes in the blood before immunization of Th male and female mice are, to some extent, similar to a larger or smaller increase in their number in 7 days with the following decrease in their number (Figures 4B and 5B). After immunization of female mice, there is a very slight increase in CD8 lymphocytes with a temporary plateau when male mice show a sharp increase in the number of these cells up to day 22 with their subsequent decrease (Figure 5B). A maximal 2-fold difference (P < 0.05) in the number of CD8 cells in immunized female and male mice is observed 22 days after their treatment. The characters of the in-time changes in CD8 cells in the thymus of Th male and female mice before immunization is somewhat close to that for these lymphocytes changes in the bone marrow (Figure 5C) and CD4 cells in the thymus (Figure 4C). The curve corresponding to overtime changes in CD8 in
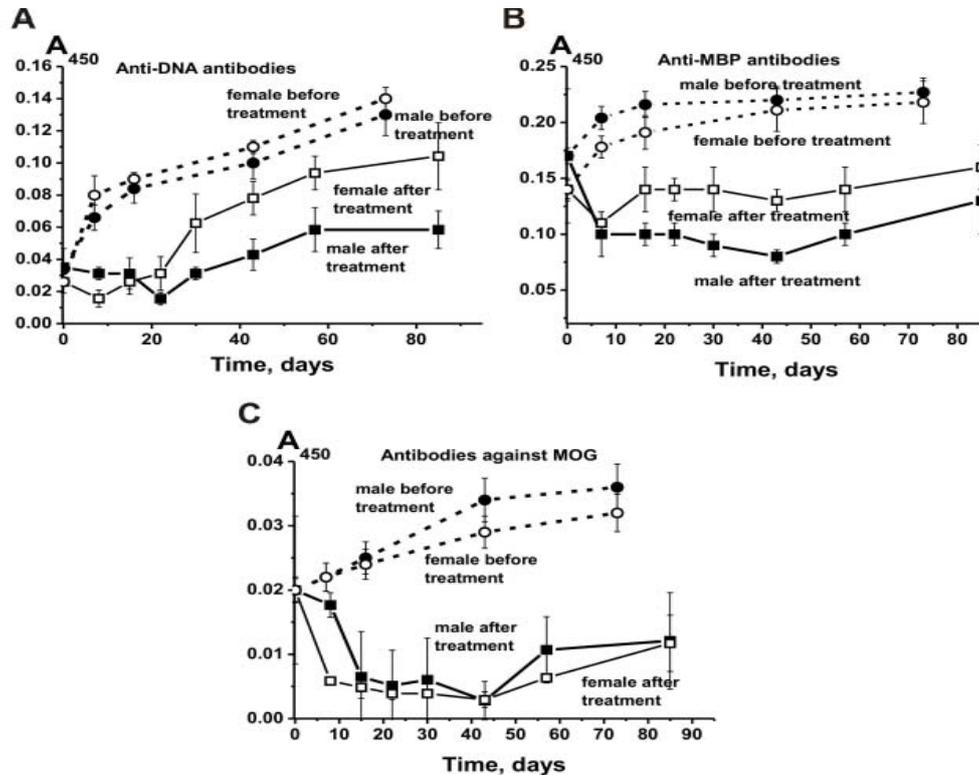
Figure 6: In time changes in the relative concentration of Abs to DNA (A), MBP (B), and MOG (C) in Th mice untreated and treated with MOG. All antibody concentrations were measured by ELISA. The error in the determination of the concentration from three independent experiments in the case of each mouse of every group (7mice) did not exceed 7-10%.

the thymus of male mice (Figure 5C) after their immunization almost repeats that for the bone marrow (Figure 5A). At the same time, the patterns of changes in CD8 lymphocytes in the thymus and bone marrow of female mice treated with MOG are very different (Figures 5A and 5C). In contrast to the continuous decrease in time of the number of these cells in the bone marrow of immunized female mice, a sharp increase in their content from 15 to 22 days is observed in the thymus (Figure 5C). Interestingly, the patterns of changes in the number of CD8 cells in the thymus in female and male mice before and after their immunization are very similar (Figure 5C). The time-dependent curves of the number of CD8 lymphocytes during the spontaneous development of EAE in the spleen of Th male and female mice are different (Figure 5D). Female mice show a constant decrease in these lymphocytes, reaching a plateau at about day 40. In male mice, there is a remarkable increase in the number of CD8 cells up to 15-20 days with their subsequent decrease. Immunization of Th male mice leads in the spleen to a sharp decrease in the number of CD8 cells with a noticeable increase in 30 days (Figure 5D). The characters of the changes in time of the relative number of CD8 (Figure 5D) as well as CD4 (Figure 4D) in the spleen of female mice are very complex. In both cases, there is a temporary increase in the relative number of CD8 and CD4 cells at ~30 and 60 days. The nature of the changes in CD8 lymphocytes in lymph nodes is very similar for male and female mice, both before and after their immunization with MOG (Figure 5E). During the spontaneous development of EAE, male and female mice in overall show an almost smooth increase in the number of these cells over time. After immunization of male and female mice,

there is a sharp increase in the relative number of CD8 lymphocytes to day 22 with their subsequent decrease (Figure 5E). Thus, the characters of the changes in CD4 and CD8 cells in all organs of Th male and female mice are very complex. The greatest differences in the number of CD4 and CD8 cells are observed in the bone marrow of male and female mice after their immunization with MOG (Figures 4A and 5A). In addition, there was observed a significant difference in the dependencies of overtime changes in CD8 cells in male and female mice after their immunization with MOG (Figure 5B). The most complex and unlike other organs character of changes in CD4 and CD8 lymphocytes was found in the spleen of female mice (Figures 4D and 5D). Initial symptoms of the development of EAE in MOG-induced C57BL/6 mice (onset) is usually observed 6-8 days after their immunization, while the acute stage of the EAE usually occurs at 17-20 days after the immunization and after 30-40 days begins remission stage [29-32]. Interestingly, after immunization with MOG in nearly all organs of Th male and less often in female mice similar to C57BL/6 mice; there was a sharp increase in the relative average number of CD4 and CD8 lymphocytes in the period from 7 to 23 days corresponding to the onset and acute stage (Figures 4 and 5). In the period corresponding to the mouse remission stage (after 30-40 days), depending on the organ, there might be a remarkable decrease or an increase in the relative number of CD4 and CD8 lymphocytes.

The relative content of antibodies against proteins and DNA

The blood of different healthy mammals usually contains autoantibodies against DNA, RNA, and various proteins in low
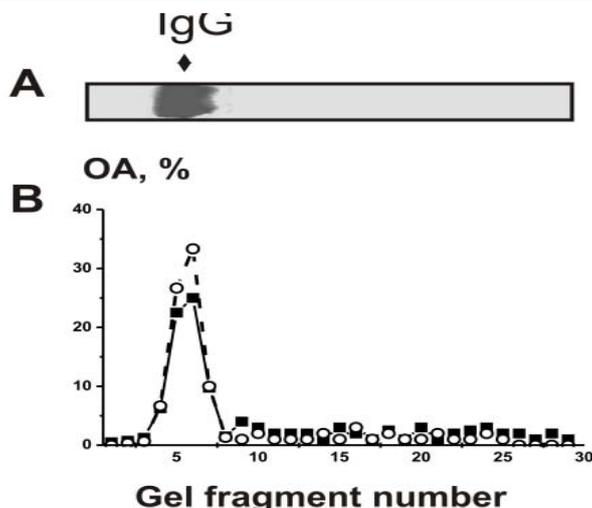
Figure 7: The homogeneity analysis by SDS-PAGE of 12μg IgGmix under non-reducing conditions (A); silver staining; panel A shows the position of IgGs. The relative activities (RA, %) in the hydrolysis of DNA (o) and MBP (■) by eluates of gel fragments were estimated using the extracts of gel fragments (2-3mm) (B). Complete hydrolysis of these substrates after their incubation with eluates for 24h of was taken for 100% (A). The errors of the RAs determinations from two independent experiments did not exceed 7-10%.
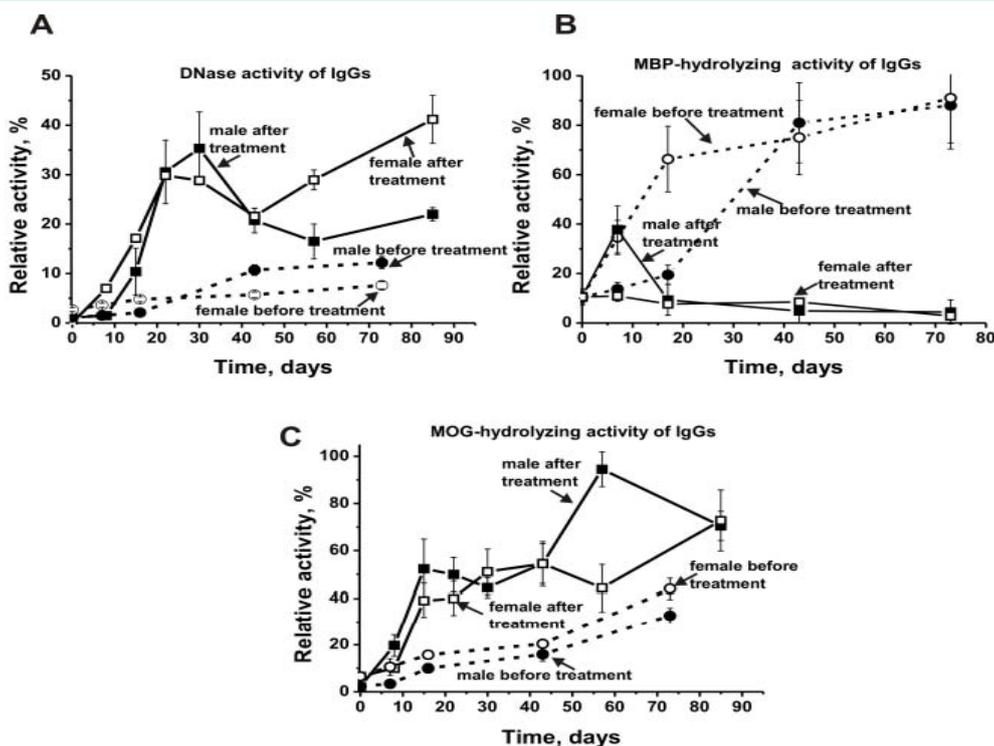

Figure 8: In time changes in the relative average (RAs) activities of Th IgGs before and after mice treatment (immunization) with MOG in the hydrolysis of DNA (A), MBP (B), and MOG (C); RA of mice IgGs corresponding to 7 individual mice of every group. The rror in the individual RA determination for every mouse of each group does not exceed 7– 942 10%.

concentration [13-17]. The concentrations of auto-Abs to DNA in blood sera of non-autoimmune BALB and CBA mice (at 3-15 months of age) as well as healthy MRL-lpr/lpr mice (at 2-3 months of age) are low; 0.03-0.04 A_{450} units [9-11]. In sera of EAE prone C57BL/6 mice, the concentrations of anti-DNA Abs are higher (~0.11 A_{450} units), and during spontaneous development of pathology, it increased slowly over 1.5-2 months up to 0.15 A_{450} units [7,8,34,35] (Supplementary

Figure S3). During 73 days of spontaneous development of EAE, the anti-DNA Abs concentration in Th mice increased 5.3- and 3.8-fold ($P < 0.05$) for female and male mice, respectively (Figure 6A). The treatment of Th mice with MOG led to a significant and comparable decrease in anti-DNA Abs concentration in sera of male and female mice during the onset and acute phase (7-23 days) of EAE (at 14 days 3.6-4.0-fold, $P < 0.05$) (Figure 6A). However, then, during the period

of remission (> 30 days), the concentration of anti-DNA antibodies was slowly increased, he difference in the concentration of anti-DNA Abs before and after

Immunization of mice with MOG duringt he entire analysis period was statistically significant, P < 0.05. Interestingly, unlike Th mice, immunization with MOG of C57BL/6 male mice did not lead not to the decrease but to an increase in the concentration of anti-DNA in the blood sera (Supplementary Figure S3). We evaluated the changes over time in the concentration of Abs against MBP and MOG using homogeneous preparations of polyclonal IgGs isolated from the sera of male and female mice. The relative concentration of anti-MBP during 73 days of spontaneous development of EAE increased 2.3-fold in females and 1.8-fold in male mice (P < 0.05). As in the case of anti-DNA auto-Abs, immunization of male and female mice with MOG led to a sharp decrease in the concentration of antibodies against MBP starting the first seven days after mice immunization (at 7 days 1.6-2.0-fold, P < 0.05) (Figure 6B). Wherein, after immunization, a statistically significant difference was observed between the anti-MBP IgGs concentrations in female and male mice (P = 0.02). As shown earlier, the blood of C57BL/6 mice, even at 3 months of age, contains Abs against MBP and their concentrations increase during spontaneous but much faster after male immunization with MOG (Supplementary Figure S3) [7,8,34,35]. During the spontaneous development of EAE in Th male and female mice, auto-Abs against MOG increased almost linearly from 0.02 to 0.032-0.036 A_{450}, P < 0.05 (Figure 6C). It was reasonable to expect that immunization of mice with external MOG will lead to a sharp increase in the concentration of antibodies against this peptide. However, we obtained an unexpected result. The immunization of mice with MOG peptide led to a very powerful suppression of the formation of antibodies against this peptide (from 7 to 40 days) in both male and female mice (Figure 6C) with the statistically significant difference before and after mice treatment, P = 0.005 and 0.01, respectively. During spontaneous development of EAE in C57BL/6 male mice, as well as in Th male mice, a gradual, almost linear increase in the concentration of antibodies against MOG was observed. After C57BL/6 male mice immunization in contrast to Th mice, there was observed a higher increase of anti-MOG Abs in comparison with spontaneous development of EAE (Supplementary Figure S3). Thus, in contrast to non-autoimmune mice, the concentration of antibodies against DNA, MBP, and MOG increases during spontaneous development of EAE over time in Th male and female mice, as is the case for other animals predisposed to develop spontaneous autoimmune diseases [7,8,34,35]. The main difference in the production of auto-Abs in autoimmune prone Th and C57BL/6 mice is that immunization with MOG leads to a sharp decrease in the concentration of Abs against DNA, MBP, and MOG in Th mice, while there is a sharp increase in their concentration at the onset and acute phase of the disease in C57BL/6 mice.

Criteria analysis of catalytic activities of antibodies

Previously, it has been demonstrated that in contrast to non-autoimmune mice (BALB and CBA), IgG antibodies from the blood of EAE prone C57BL/6 mice [7,8,34,35] already at the age of 3 months possess the catalytic activity, and they are able to hydrolyze DNA, MBP and MOG. The level of catalytic activity of IgG-abzymes of C57BL/6 mice in the hydrolysis of these substrates is continuously increasing during spontaneous and to a greater extent, after MOG-

induced development of EAE [7,8,34,35].To obtain electrophoretically homogeneous IgG preparations deprived of impurities of any classical enzymes, we have developed a universal technique. It includes affinity chromatography on Protein G-Sepharose of sera proteins in conditions removing nonspecifically bound components [7,8,34,35]. The purified IgGs are then additionally subjected to FPLC gel filtration in an acidic buffer (pH = 2.6). The homogeneity of IgGs was shown in this article (Figure 7A) as in all previous publications by SDS-PAGE with silver staining [7,8,34,35]. To show that DNA- and different proteins-hydrolyzing activities are the own properties of antibodies, and they do not contain co-purified canonical enzymes, we used several known very strict criteria [7,8,34,35]. It was shown that IgGs from sera of Th mice obtained using the described approach do not contain impurities of any canonical DNases and proteases. A more detailed description of the method of IgGs antibodies purification from sera of mice and data on the analysis of the affiliation of enzymatic activities directly to antibodies are given in Supplementary Methods (part 2: "IgG purification"). To prove the belongings of enzymatic activities to Th mice antibodies, a mixture of IgGs was used for the analysis of catalytic activities (Figure 7B). After IgGs SDS-PAGE (part 3 "SDS- PAGE analysis of catalytic activities"), it was shown that Th mice antibodies do not contain any impurities of canonical enzymes, and they may be used to evaluate their relative activity in the hydrolysis of DNA and proteins.

Time-dependent changes in IgGs catalytic activities

It has been shown that abzymes that hydrolyze DNA and proteins are absent in the blood of non-autoimmune BALB and CBA mice. The appearance of abzymes possessing various enzymatic activities is shown as a very important marker of onset and development for various AIDs [7-11,34,35]. Healthy humans do not usually demonstrate catalytic activities of Abs [13-17]. The blood sera of SLE-prone MRL-lpr/lpr male and female mice contain Abs-abzymes possessing DNA-, ATP- and oligosaccharides-hydrolyzing activities [7,8,34,35]. EAE prone C57BL/6 mice show a gradual and almost linear 6.8-fold increase in DNase activity during spontaneous development of this pathology. At time zero, DNA-hydrolyzing activity of IgGs in Th female mice was approximately by a factor of 2.5 higher than that for male mice (P < 0.05) (Figure 8A). During spontaneous development (73 days) of EAE by Th mice, the DNase activity of female mice Abs was increased 2.8-fold, while for male mice, IgGs activity was risen by a factor of 11 (P < 0.05) (Figure 8A). The immunization with MOG of male mice led to an increase in this activity today 30 by a factor of 32, while for female mice 11-fold, (P < 0.05) (Figure 8A). The DNase activity of female antibodies at 83 days after immunization was 2 times higher than that from the blood of males (P < 0.05). Approximately the same situation was found earlier for C57BL/6 male mice; immunization with MOG led to an increase in activity 25-fold at about day 20 after immunization of mice (Supplementary Figure S4). It should be noted that immunization of C57BL/6 male mice leads to a strong increase in the concentration of anti-DNA antibodies and to a parallel increase in their activity in DNA hydrolysis. At the same time, at a sharp decrease in the concentration of antibodies against DNA in Th mice (Figure 6A), there is a very strong increase in their activity in the splitting of DNA (Figure 8A). The relative activity of IgGs from Th male and female mice at zero time in the 688 hydrolysis of MBP was nearly the same (Figure 8B). There was, however, a strong slowdown in the growth of abzyme MBP-

hydrolyzing activity in sera of males compared to females during the spontaneous development of EAE (Figure 8B). In male mice after immunization, there was a sharp increase in the hydrolysis of MBP antibodies at 7 days. But then, after 20 days, there was a decrease in MBP-hydrolyzing activity in male and female mice (Figure 8B). Finally, at 73 days after immunization, the activity of antibodies from the serum of male and female mice was 23-26 times lower than before their treatment with MOG after spontaneous development of EAE (P < 0.05). The profiles of changes in the activity of antibodies from the blood of female and male mice in the hydrolysis of MOG before as well as after immunization of mice are very similar (Figure 8B). The increase over time in the relative concentrations of Abs against MBP in male and female mice during spontaneous development of EAE correlates with the increase in the activity of abzymes hydrolyzing MBP (Figures 6B and 8B). At the same time, a keen decrease in the concentration of antibodies against MOG (Figure 6C) after the immunization was inversely related to the significant increase in the relative activity of these antibodies in the hydrolysis of MOG (Figure 8C). Before and after immunization of C57BL/6 male mice, there were parallel increases in the concentration of anti-MOG antibodies and their activities in MOG hydrolysis (Supplementary Figures S3 and S4). Thus, some noticeable differences are apparent for male and female mice, not only in the dependencies of changes over time in differentiation profiles of stem cells (Figure 1) and patterns of changes in lymphocyte proliferation in different organs (Figures 2-4), but also in relative concentrations of Abs against DNA, MBP, and MOG, as well as activities in their hydrolysis (Figures 6 and 8).

Discussion

The spontaneous achievement of SLE in MRL-lpr/lpr and EAE in C57BL/6 mice is significantly accelerated by mice treatment with DNA and MOG, respectively. The development of SLE in MRL-lpr/lpr and EAE in C57BL/6 mice are characterized by very similar changes in the HSCs differentiation profile and the beginning of the production of different abzymes at the onset and acute phases of EAE and SLE [7-11,34,35]. The activated myelin-reactive B and T cells are both important for MS pathogenesis in mammals [1-3]. Therefore, it was interesting to analyze the changes of pathology development important parameters during the spontaneous and MOG-accelerated development of EAE in Th mice, demonstrating a T lymphocyte response [33]. The main difference in the differentiation profiles of HSCs during spontaneous development of EAE in Th male and female mice was revealed for CFU-E, CFU-GEMM and T cells. However, the differentiation of BFU-E, CFU-GM and B cells was, to some extent, similar (Figure 2). After mice immunization with MOG, a certain similarity in the differentiation was observed for BFU-E, CFU-E, CFU-GEMM and B-cells, but it was reverse directions for CFU-GM colonies and T cells (Figure 2). Some differentiation profiles of HSCs in male C57BL/6 mice (Supplementary Figure S2) in overall were different in comparison with Th mice (Figure 2). After immunization of mice with MOG in nearly all organs of Th male and female mice similar to C57BL/6 mice, was observed a sharp increase in the relative number of lymphocytes in the period from 7 to 23 days corresponding to the onset and acute stages of pathology (Figures 4 and 5). Despite the differences between male and female mice in profiles of HSCs differentiation and lymphocyte proliferation in various organs, in both cases, these processes lead to the production

of auto-Abs against DNA, MBP, and MOG, including abzymes hydrolyzing these substrates (Figures 6 and 8). Many parameters the development of EAE in C57BL/6 mice (Supplementary Figures S1-S4) are significantly different in comparison with Th mice. Interestingly, in the case of C57BL/6 mice, there is some correlation between the growth of antibody concentrations against DNA, MOG and MBP, and the parallel increase in the efficiency of these substrates hydrolysis up to about 20-30 days (Supplementary Figures S3 and S4). At the same time several dependencies showing changes in concentrations of antibodies against DNA, MBP and MOG in Th mice do not correlate with those characterizing hydrolysis of these substrates (compare Figures 6 and 8). It was shown that due to the extreme diversity of abzymes hydrolyzing different antigens, specific stages of AIDs development might be characterized by the production of various auto-Abs without and with low or high catalytic activities [11-17]. It is reasonable to suggest, therefore, that after immunization of Th mice with MOG, a parallel decrease in the concentration of the auto-Abs without catalytic activity takes the increase in the concentration of abzymes hydrolyzing these antigens. It should be assumed that significant differences in immunoregulation in women and in men can occur not only at the level of stem cell differentiation and production of abzymes but also in the expression of immune-related microRNAs. For example, it was shown that in patients with multiple sclerosis, miR-379-5p and miR-223-3p were upregulated only in men [41,42]. Increased expression of miR-127-3p, miR-431, miR-379, miR-381, miR-376c, miR-656, and miR-410 has also been revealed only in males but not in female analyzed patients with relapsing-remitting multiple sclerosis [41,42]. It cannot be ruled out that the development of MS in men and women may differ with respect to different changes in any other parameters.

Conclusion

Here, the analysis of several important specific parameters characterizing spontaneous and MOG-induced development of EAE in Th mice with a T lymphocytes response and their comparison with those for C57BL/6 mice with T- and B-response was carried out for the first time. Despite the fact that the significant differences in the studied parameters in Th and C57BL/6 mice, the development of EAE, in both cases, is associated with a change in the bone marrow stem cell differentiation profile, an increase in the proliferation of lymphocytes in various organs, and the production of catalytic antibodies that are harmful to mice.

Acknowledgement

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