

RESEARCH ARTICLE

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Evaluation of Some Blood Parameters in The Experimental Autoimmune Encephalomyelitis Mouse Model

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Abstract

Multiple sclerosis (MS) is a chronic neuroinflammatory demyelinating disorder of the central nervous system with unclear exact etiology. The experimental autoimmune encephalomyelitis (EAE) model in C57BL/6 mice is the most common animal model for MS sharing many clinical and pathophysiological features to expand our knowledge on the pathophysiology of the disease and to develop novel treatment strategies. The current study was designed to determine hematological parameters and plasma total protein (TP) and albumin (ALB) levels in EAE-induced C57BL/6 mice to help researchers working on the EAE animal model.

EAE was induced with myelin oligodendrocyte glycoprotein (MOG35-55) peptide in the female C57BL/6 mice. The EAE clinically caused paralyzed tail, hind limb paresis, and uncoordinated movement in the mice. The EAE-induced mice hematologically had a statistically significant mild increase in white blood cell (WBC) count without altering neutrophil-lymphocyte ratio but no change in vital hematological parameters such as red blood cell count, packed cell volume, and hemoglobin level. Moreover, the EAE led to an increase in the plasma TP level and attenuation in plasma ALB level in the mice.

In conclusion, our findings show that the EAE model in mice might not cause any significant change hematologically, except a slight increase in the WBC count, and might produce changes in the plasma protein level. As the findings of the current study, the EAE-induced blood parameter effects could consider understanding the pathophysiology of the disease and developing a novel therapeutic approach for the disease.

Keywords: Experimental autoimmune encephalomyelitis mouse model, Hemogram, Plasma total protein, Plasma Albumin

Introduction

Multiple sclerosis (MS) as a central nervous system (CNS) disorder is not fully understood due to its complex immunopathogenetic mechanisms.¹⁻³ Moreover, MS may cause a different path pathology in the white and grey matters of CNS because of its complex mechanism.^{4,5} Previous studies mostly focused on investigating white matter damage, in which pathological features include inflammatory infiltration and associated blood-brain barrier disruption, demyelination, and glial scarring, as well as axonal damage within the lesions and in the normal-appearing white matter.⁶⁻⁹ To

date, animal models such as experimental viral infection¹⁰ and experimental autoimmune encephalomyelitis (EAE)¹¹⁻¹³ have been generated to understand the disease.

EAE has been the most widely used model produced by applying immunization with myelin protein or peptides derived from myelin protein in susceptible animals. Myelin basic protein,¹⁴⁻¹⁶ calcium-binding astrocytic protein S100- β ,¹⁷ proteolipid protein,¹⁸ minor components including myelin-associated glycoprotein,^{19,20} myelin-associated oligodendrocyte basic protein,²¹ or myelin oligodendrocyte glycoprotein (MOG)²²⁻²⁵ has been used as the antigen to generate the EAE model up to now. In the EAE model

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in the rodents, it is required to use precise combinations of antigen and animal strain to display the clinical or pathological symptoms of the disease, such as lesion topography, cellular composition of inflammatory infiltrate, and clinical profile.²⁶⁻²⁹

The MOG35–55-induced EAE model has been the most commonly used one to study early pathological and clinical changes and to develop a new therapeutic approach for the disease.³⁰⁻³² This EAE model generates a chronic progressive course in the C57BL/6 mice.³³ Therefore, C57BL/6 mice were the most chosen animal strain to develop the EAE model. Although the studies reported so far are mainly based on the pathologic mechanism of EAE and identifying new therapeutic approaches, there are no studies on hematological parameters of EAE-induced animals. This study aimed to determine how the hematological parameters and plasma total protein and albumin levels are affected in EAE-induced C57BL/6 mice to assist researchers working on the mechanism or novel therapeutic approach of EAE.

Materials and Methods

Female 8 adults C57BL/6 mice (8 weeks old, 16–20 g) were purchased from Animal House in the Department of Molecular Biology and Genetics at Bilkent University. The mice were housed as four mice per cage for one-week acclimation and quarantine period before starting the experiments in a room with a proper care environment (20–22 °C; 60–70% humidity; 12 h light/dark cycle). After the period, the mice were randomly divided into two groups: the control and the EAE groups. Bursa Uludag University, the local animal ethical committee, approved all experimental procedures (2021-05/08).

MOG35-55 peptide (Immunostep, 181214-6) was first dissolved as a 200 µg peptide in 100 µl ddH₂O to develop an EAE mice model. And then, the dissolved MOG35–55 peptide was emulsified in 100 µl of complete Freund's adjuvant (Sigma-Aldrich, F5506), containing Mycobacterium tuberculosis (BD 231141) (4 mg/ml) to inject the animals as the final MOG35–55 peptide solution. The EAE group mice were subcutaneously injected twice MOG35–55 peptide solution as 100 µl for each the left and right flank side. Just after the MOG35–55 peptide solution injection, Pertussis toxin (Gibco, PHZ1174) in ddH₂O (400 ng/200 µl) was intraperitoneally injected. The Pertussis solution was reinjected 48 h later in the same way. The mice in the control group were injected with PBS instead of the MOG35–55 peptide or Pertussis toxin in the same way and the volume. Each group consisted of 4 mice. The EAE clinical score and body weight were recorded every two days for 20 days after the first injection day for the mice. As report-

ed by Bittner et al.,³³ clinical scores were given to follow disease progression, as 0, no symptoms; 1, partially limp tail; 2, paralyzed tail; 3, hind limb paresis and uncoordinated movement; 4, one hind limb paralyzed; 5, both hind limbs paralyzed; 6, hind limbs paralyzed and weakness in forelimbs; 7, hind limbs paralyzed and one forelimb paralyzed; 8, hind limbs paralyzed, both forelimbs paralyzed; 9, moribund; 10, death. Through the experiment, it was paid attention that the animals, especially EAE-induced ones, had access to feed and water.

The experiment was terminated on the 20th day after the first injection. At the end of the experiment, the 250 µl of the blood sample for each mouse was collected in the tubes with EDTA by decapitating under Sevoflurane (2-4 %/100 % O₂) anesthesia. The hemogram and plasma total protein (TP) and albumin (ALB) levels were immediately measured by using Abbott CELL-DYN Ruby Hematology System and Abbott ARCHITECT c16000 Clinical Chemistry System (Illinois, USA), respectively, from the blood samples. In the hemogram, the red blood cell count (RBC, x10⁶/mm³), the packed cell volume (PCV, %), the hemoglobin level (Hb, g/dl), the mean corpuscular volume (MCV, µm³), the mean corpuscular hemoglobin (MCH, pg), the mean corpuscular hemoglobin concentration (MCHC, g/dl), the red blood cell distribution width (RDW, %), the white blood cell count (WBC, x10³/mm³), and the neutrophil-lymphocyte ratio (NLR, %) were measured.

The data in the table are given as mean with standard error (SEM) within *p < 0.05 significance level. Student's t test performed the statistical comparison between control and EAE groups.

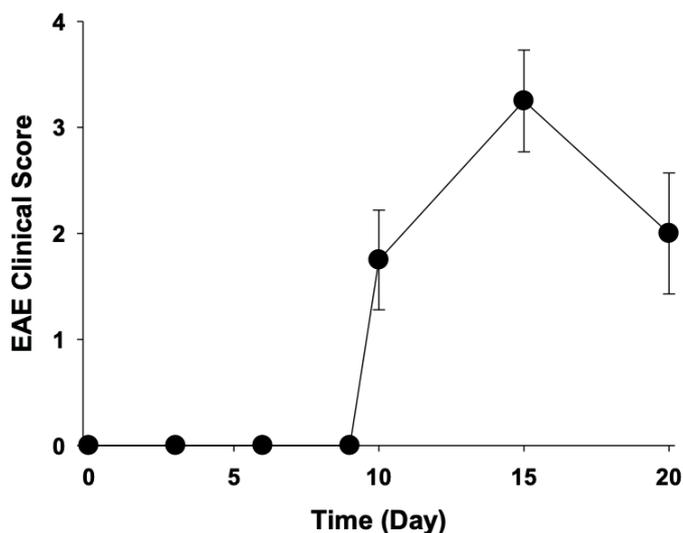
Results

The mice's body weight did not change with EAE induction (Table 1). While animals in the control group did not show any clinical symptoms, the EAE-induced animals had the clinical symptom score started on day 10 after the first injection (1.75 ± 0.47) and continued throughout the experiment (Fig. 1). The clinical symptom score in the EAE-induced animals peaked on day 15 after the first injection (3.25 ± 0.48), and it was 2.00 ± 0.57 at the end of the experiment (20th day of the first immunization) (Fig. 1).

Table 1. The effect of EAE on the body weight of mice.

	Body weight (g)	
	Control Group	EAE Group
The start of the experiment	16.00 ± 0.41	16.00 ± 0.70
The end of the experiment	17.42 ± 0.84	17.50 ± 1.04

The body weight of the mice was recorded every 2 days throughout the experiments. The table shows the body weight of the control and EAE mice at the start and the end of the experiment.



EAE induction in the mice did not alter RBC, PCV, Hb Figure 1. The graph shows the clinical scores after the immunization over 20 days. EAE was induced to the mice as described in the material and method section. Clinical symptoms were scored as reported by Bittner et al.,³³. Data are shown as the mean and standard error of the mean (n = 4).

Table 2. The effect of EAE on some blood parameters of mice.

Parameters	Control Group	EAE Group
RBC (x10 ⁶ /mm ³)	6.926 ± 0.172	7.073 ± 0.196
PCV (%)	34.12 ± 0.99	33.83 ± 1.38
Hb (g/dl)	10.406 ± 0.342	10.507 ± 0.632
MCV (µm ³)	49.22 ± 0.24	48.27 ± 0.83
MCH (pg)	15.0 ± 0.2	14.2 ± 0.6
MCHC (g/dl)	30.46 ± 0.21	29.67 ± 0.73
RDW (%)	9.62 ± 0.50	9.71 ± 0.15
WBC (x10 ³ /mm ³)	1.98 ± 0.35	2.98 ± 0.58*
NLR (%)	0.069 ± 0.013	0.063 ± 0.015
TP (g/dl)	35.1 ± 1.81	39.2 ± 2.05*
ALB (g/dl)	17.53 ± 1.21	13.04 ± 1.50*

At the end of the experiment, 250 µl of blood samples from each animal were collected in tubes containing EDTA by decapitating under anesthesia. The parameters were measured by using the Abbott CELL-DYN Ruby Hematology and Abbott ARCHITECT c16000 Clinical Chemistry Systems. The data was given as mean ± SEM of four rats. Statistical analysis was performed using Student t test. *p < 0.05, significantly different from the values of the control group.

level, MCV, MCH, MCHC, and RDW compared to control animals without EAE induction (Table 2). However, the EAE induced mice had a mild but statistically significant (p < 0.05) increase in total WBC count without changing NLR (Table 2). Moreover, when compared to control mice,

EAE induction in the animals caused a statistically increase in plasma TP level (p < 0.05) while a statistically decrease in plasma ALB level (p < 0.05; Table 2).

Discussion and Conclusion

Here, we evaluated clinical, hematological, and plasma TP and ALB levels in MOG35–55-induced EAE in the C57BL/6 mouse strain, the most commonly used host for gene deletion studies. Clinically the EAE caused the tail and hind limb paresis and uncoordinated movement in the mice. These clinical symptoms were consistent with the clinical symptoms obtained in the MOG35–55-induced EAE model protocol previously described by Bittner et al.³³ As described by Bittner et al.³³; the EAE clinical symptoms began to appear on the 10th day of inducing EAE in mice and reached a peak level around the 15th day. Clinical observations obtained by EAE induction in the present study were consistent with the previously reported results in terms of timetable and symptoms, confirming that the mice can be induced with EAE in the study.^{27,33,34}

The EAE did not affect the vital blood parameters such as RBC and RBC-related blood parameters, although it caused the clinical symptoms. All critical parameters of the EAE-induced animals about RBC-dependent oxygenation, such as RBC count, PCV, Hb level, MCV, MCH, MCHC, and RDW, were similar with control group animals. These findings can be accepted as indicating that EAE induction applied in the present study is working without creating a life-threatening in mice. No many reports on RBC in the EAE model. It was only reported that RBC was extravasated into the CNS in the EAE animal model.^{35,36} Also, a difference in RDW in chronic MS patients according to healthy ones was determined.³⁷

The current findings showed that EAE-induced mice had a slight increase in WBC count and caused a not statistically significant decrease in NLR. The differential ratio of WBC and the total count of WBC are widely-used biomarkers of systemic inflammation. Findings of neutrophilia and lymphopenia in response to bacterial infections or cancer have led to the investigation of the NLR as a biomarker that might reflect systemic inflammation better than the neutrophil or lymphocyte count alone.³⁸ It was reported that neutrophils are one of the significant sources of inflammatory cells to initiate EAE in the acute phase.³⁹ The decrease in NLR might be due to infiltration of neutrophils in EAE induction. The increase in WBC count might be due to the immune system's reaction against brain-specific antigens, which are MOG35-55 and pertussis toxin solution used in the protocol. As a result of the response, activated myelin-specific leukocytes infiltrate into the CNS.⁴⁰

In the current study, an increase in TP but a decrease in

ALB level with EAE induction in the mice was observed. It was previously reported that a characteristic pattern of protein changes was observed both in the blood serum and in the cerebrospinal fluid in the patients with MS.⁴¹ The previous electrophoretically study revealed that MS patients had decreased ALB and ALB-globulin ratio values with extra total protein in cerebrospinal fluid.⁴¹ EAE-caused blood-brain barrier leakage results in the extravasation of plasma proteins, especially ALB, into the CNS. Besides accessing the CNS following blood-brain barrier leakage, ALB was also produced by microglia cells.⁴² The increase in globulin level caused by EAE as well as injection of MOG to form the model might cause the observed increase in TP level. The leakage of ALB into the CNS seems to be the underlying cause of the observed decrease in ALB level.

As a result, the induction of EAE in mice caused clinical symptoms, including the paralyzed tail, hind limb paresis, and uncoordinated movement. Moreover, the EAE increased WBC and plasma TP level and decreased plasma ALB level without changing vital hematological parameters. The assessment of The EAE mouse model commonly used, as MS-like-disease, in terms of hematologic parameters and plasma TP and albumin levels might present useful data for researchers working on pathogenesis or novel therapeutic approaches of the MS disease.

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References

- Kieseier BC, Hemmer B, Hartung HP. Multiple sclerosis - novel insights and new therapeutic strategies. *Curr. Opin. Neurol.* 2005;18:211-220.
- Prat A, Antel J. Pathogenesis of multiple sclerosis. *Curr. Opin. Neurol.* 2005;18:225-230.
- Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* 2008;31:247-269.
- Kutzelnigg A, Faber-Rod JC, Bauer J, Lucchinetti CF, Sorensen PS, Laursen H, Stadelmann C, Bruck W, Rauschka H, Schmidbauer M, Lassmann H. Widespread demyelination in the cerebellar cortex in multiple sclerosis. *Brain Pathol.* 2007;17:38-44.
- Lassmann H, Bruck W, Lucchinetti CF. The immunology of multiple sclerosis: an overview. *Brain Pathol.* 2007;17:210-218.
- Bjartmar C, Trapp BD. Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. *Curr. Opin. Neurol.* 2001;14:271-278.
- Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997;120:393-399.
- Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neuritis, apoptotic neurons and reduced inflammation in cortical multiple sclerosis lesions. *Ann. Neurol.* 2001;5:389-400.
- Frohman EM, Racke MK, Raine CS. Multiple sclerosis - the plaque and its pathogenesis. *N. Engl. J. Med.* 2006;354:942-955.
- Nelson ALA, Beiber AJ, Rodriguez M. Contrasting murine models of MS. *Int. MS J.* 2004;11:95-99.
- Ercolini AM, Miller SD. Mechanisms of immunopathology in murine models of central nervous system demyelinating disease. *J. Immunol.* 2006;176:3293-3298.
- Gold R, Linnington C, Lassmann H. Understanding the pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 2006;129:1953-1971.
- Lassmann H. Models of multiple sclerosis: new insights into pathophysiology and repair. *Curr. Opin. Neurol.* 2008;21:242-247.
- Einstein ER, Robertson DM, Dicaprio JM, Moore W. The isolation from bovine spinal cord of a homogeneous protein with encephalitogenic activity. *J. Neurochem.* 1962;9:353-361.
- Hashim GA, Sharpe RD, Carvalho E, Stevens LE. The development of experimental allergic encephalomyelitis with immunizing doses of myelin basic protein. *Proc. Soc. Exp. Biol. Med.* 1975;149:646-651.
- Martenson RE, Deibler GE, Kies MW. Myelin basic proteins of the rat central nervous system. Purification, encephalitogenic properties and amino acid compositions. *Biochem. Biophys. Acta* 1970;200:353-362.
- Kojima K, Wekerle H, Lassmann H, Berger T, Linnington C. Induction of experimental autoimmune encephalomyelitis by CD4+T cells specific for an astrocyte protein, S100 β . *J. Neural. Transmem. Suppl.* 1997;49:43-51.
- Kuchroo VK, Sobel RA, Laning JC, Martin CA, Greenfield E, Dorf ME, Lees MB. Experimental allergic encephalomyelitis mediated by cloned T cells specific for a synthetic peptide of myelin proteolipid protein. Fine specificity and T cell receptor V beta usage. *J. Immunol.* 1992;148:3776-3782.
- Morris-Downes MM, McKormak K, Baker D, Sivaprasad D, Natkunarajah J, Amor S. Encephalitogenic and immunogenic potential of myelin-associated glycoprotein (MAG), oligodendrocyte specific glycoprotein (OSP) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) in ABH and SJL mice. *J. Neuro-*

- immunol. 2002;122:20-33.
20. Weerth S, Berger T, Lassmann H, Linington C. Encephalitogenic and neuritogenic T cell responses to the myelin-associated glycoprotein (MAG) in the Lewis rat. *J. Neuroimmunol.* 1999;95:157-164.
 21. Maatta JA, Kaldman MS, Sakoda S, Salmi AA, Hinkkanen AE. Encephalogenicity of myelin-associated oligodendrocytic basic protein and 2',3'-cyclicnucleotide 3'phosphodiesterase for BALB/c and SJL mice. *Immunology* 1998;9:383-388.
 22. Kerlero de Rosbo N, Kaye JF, Eisenstein M, Mendel I, Hoefftberger R, Lassmann H, Milo R, Ben-Nun A. The myelin-associated oligodendrocytic basic protein region MOBP15-36 encompasses the immunodominant major encephalitogenic epitope(s) for SJL/J mice and predicted epitopes(s) for multiple sclerosis-associated HLA-DRB1*1501. *J. Immunol.* 2004;173:1426-1435.
 23. Laman JD, van Meurs M, Schellekens MM, de Boer M, Melchers B, Massacesi M, Lassmann H, Claassen E, 't Hart B. Expression of accessory molecules and cytokines in acute EAE in marmoset monkeys (*Callithrix jacchus*). *J. Neuroimmunol.* 1998;86:30-45.
 24. Mendel I, Kerlero de Rosbo N, Ben-Nun A. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. *Eur. J. Immunol.* 1995;25:1951-1959.
 25. Raine CS, Cannella B, Hauser SL, Genain CP. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case for antigen-specific antibody mediation. *Ann. Neurol.* 1999;46:144-160.
 26. Berger T, Weerth S, Kojima K, Linington C, Wekerle H, Lassmann H. Experimental autoimmune encephalomyelitis: the antigen specificity of T lymphocytes determines the topography of lesions in the central and peripheral nervous system. *Lab. Investig.* 1997;6:259-274.
 27. Kuerten S, Angelov DN. Comparing the CNS morphology and immunobiology of different EAE models in C57Bl/6 mice - a step towards understanding the complexity of multiple sclerosis. *Ann. Anat.* 2008;190:1-15.
 28. Schmidt S. Candidate autoantigens in multiple sclerosis. *Mult. Scler.* 1999;5:147-160.
 29. Gold R, Linnington C, Lassmann H. Understanding the pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 2006;129:1953-1971.
 30. Ayers MM, Hazelwood LJ, Catmull DV, Wang D, Kormack Q, Bernard C, Orian JM. Early glial responses in murine models of multiple sclerosis. *Neurochem. Int.* 2004;45:409-419.
 31. Pham H, Ramp A, Klonis N, Ng SW, Klopstein A, Ayers MM, Orian JM. The astrocytic response in early experimental autoimmune encephalomyelitis occurs across both the grey and white matter compartments. *J. Neuroimmunol.* 2009;208:30-39.
 32. Wang D, Ayers MM, Catmull DV, Hazelwood LJ, Bernard C, Orian JM. Astrocyte-associated axonal damage in pre-onset stages of experimental autoimmune encephalomyelitis. *Glia* 2005;51:235-240.
 33. Bittner S, Afzali AM, Wiendl H, Meuth SG. Myelin Oligodendrocyte Glycoprotein (MOG35-55) Induced Experimental Autoimmune Encephalomyelitis (EAE) in C57BL/6 Mice. *J. Vis. Exp.* 2014;86:e51275.
 34. Pham H, Doerrbecker J, Ramp AA, D'Souza CS, Gorasia DG, Purcell AW, Ayers MM, Orian JM. Experimental autoimmune encephalomyelitis (EAE) IN C57Bl/6 mice is not associated with astrogliosis. *J. Neuroimmunol.* 2011;232:51-62.
 35. Forge JK, Pedchenko TV, LeVine SM. Iron deposits in the central nervous system of SJL mice with experimental allergic encephalomyelitis. *Life Sci.* 1998;63:2271-2284.
 36. Raine CS, Barnett LB, Brown A, Behar T, McFarlin DE. Neuropathology of experimental allergic encephalomyelitis in inbred strains of mice. *Lab. Invest.* 1980;43:150-157.
 37. Peng YF, Cao WY, Zhang Q, Chen D, Zhang ZX. Assessment of the relationship between red cell distribution width and multiple sclerosis. *Medicine (Baltimore)* 2015;94:e1182.
 38. Yoon NB, Son C, Um SJ. Role of the neutrophil-lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community-acquired pneumonia. *Ann. Lab. Med.* 2013;33:105-110.
 39. Wu F, Cao W, Yang Y, Liu A. Extensive infiltration of neutrophils in the acute phase of experimental autoimmune encephalomyelitis in C57BL/6 mice. *Histochem. Cell Biol.* 2010;133:313-322.
 40. Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin. Exp. Immunol.* 2010;162:1-11.
 41. Volk BW, Saifer A, Rabiner AM, Oreskes I. Protein profile in multiple sclerosis. *AMA Arch. Neurol. Psychiatry* 1955;73:66-75.
 42. LeVine SM. Albumin and multiple sclerosis *BMC Neurol.* 2016;16:47.