Original Article

Liver Damage and Mortality in a Male Lewis Rat Model of Experimental Autoimmune Encephalomyelitis

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ABSTRACT

Background and Objectives: **Multiple sclerosis is an inflammatory disease of the central nervous system. This is due to migration of peripherally activated lymphocytes to central nervous system leading to inflammatory lesions. However, liver has an anti-inflammatory microenvironment. Myelin expression in the liver of transgenic mice suppresses inflammatory lesions within central nervous system. Considering the notion that the inflammatory events originate from periphery, we investigated if the liver was affected in an animal model for multiple sclerosis.**

Methods: **Experimental autoimmune encephalomyelitis was induced in male Lewis rats using guinea pig spinal cord and complete Freund's adjuvant. Weight, clinical score, and survival rate were evaluated for 14 days post immunization. Liver sections were taken and stained with Hematoxylin and Eosin and examined with an Olympus microscope.**

Results: **Mortality was accompanied by liver damage. Sinusoidal congestion, pycnotic nuclei within hepatocytes, hepatocyte necrosis, and severe widespread congestion along with fat accumulation within hepatocytes (fatty degeneration) were observed in liver tissue sections.**

Conclusion: **Liver damage occurs in experimental autoimmune encephalomyelitis. The perpetuation of self antigen leading to continuous migration of extrahepatically activated T cells makes an inflammatory milieu in the liver. It follows migration and development of more inflammatory cells and may paralyses tolerance inducing mechanisms. Apart from central nervous system lesion, liver injury may act as synergistic factor for debilitation and mortality.**

Key words: **Experimental Autoimmune Encephalomyelitis, Lewis Rat, Liver Damage, Mortality**

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Introduction

Multiple sclerosis (MS) is a chronic, mostly progressive, inflammatory, demyelinating, and neurodegenerative diseases of the central nervous system (CNS). It was initially assumed that inflammation was driving clinical signs in the early stages of disease, but it has been revealed that neurodegeneration and axonal damage are also abundantly present in early MS lesions (1). The real cause of the disease is unknown. On one hand, it has been attributed to dysregulation of immune system (2). Epidemiologic studies suggest that environmental factors may be more relevant than a genetic predisposition (3, 4). On the other hand, other reports are consistent with an infectious etiology. This is because of similarities between MS and post infectious autoimmune pathology (5), though no specific infectious agent has been linked to MS.

Different MS therapies have been developed to modulate the inflammatory response in the periphery and CNS. The ideal treatment of MS would be the one which induces tolerance and regulation of immune system. However, none of the current treatments have such an effect and only the few available therapeutic agents (e.g., IFN-β, glatiramer acetate, and mitoxantrone) demonstrate mild to moderate efficacy and have potential side effects. Hepatotoxicity is one of the major side effects of the above-mentioned agents (6-9). Most current reports demonstrated hepatic pathology after treatment, regarded as drug-induced hepatotoxicity. There is attempt to devise new therapeutic approaches for MS with no liver cytotoxicity (10, 11). Considering the notion that the early inflammatory events occur in the periphery triggering immune response against CNS, no report exists on whether or not liver is influenced by the inflammatory process in the CNS originating from peripherally activated immune cells. Since, current therapies cause liver injury it is notable to find out if liver injury is a consequence of therapy or is a consequence of the disease itself and occurs regardless of the therapeutic approach.

Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS. In this study influence of CNS inflammation on the liver was investigated in a Lewis rat model of EAE.

Material and Methods

Animal breeding

Male Lewis rats were originally purchased from the Darou Pakhsh, Tehran, Iran. All animal, were locally bred and kept in light- and temperature-regulated rooms at the conventional animal department of Medical Biology Research Center of Kermanshah University of Medical Sciences, western Iran. The animals were provided food and water ad libitum. All experiments were done according to Animal Care and Use Protocol of Kermanshah University of Medical Sciences.

EAE induction and clinical evaluation

Rats between 7-8 wk of age were immunized subcutaneously in the central footpad of the hind foots with 200 µl (each 100 µl) of a homogenate of equal volumes of a 50% suspension of guinea pig spinal cord (Pasture Institute, Karaj, Iran) and complete Freund's adjuvant (CFA) (Difco, Germany) (1:1, v/v), containing 4mg/ml *Mycobacterium tuberculosis* H3 RA (Difco Labs, Ditroit, MI). Each rat received 50 µg Guinea pig spinal cord and 400µg *M. tuberculosis* H3Ra.

Animals were daily weighed and clinical signs of disease were evaluated until day 14 post immunization. The signs were scored as follows: score 0, no symptoms; score 0.5, loss of tonicity of the distal portion at the tail or tail

weakness; score 1, complete tail paralysis; score 2, mild paresis of hind limbs; score 3, complete paralysis of one hind limb; score 4, bilateral hind limb paralysis; score 5, complete paralysis (tetraplegia), urinary and/or fecal incontinence, moribund state, or death. Rats with borderline scores were given a one half score.

Histological analysis

To assess the degree of inflammation, liver was dissected and fixed in 4% formalin/ paraformaldehyde for at least 48 h on day 14 post immunization (at the peak of the disease). Tissues specimens were then embedded in paraffin. Five-micrometer thick traverse sections were taken and stained with H&E and examined with an Olympus microscope.

Statistical analysis

Survival rates were illustrated by Kaplan–Meier plots. Data is presented as mean ± SEM.

Results

Clinical course, incidence, prevalence, and survival

Immunization was performed in 20 male rats. The susceptibility to EAE was 100%. Trend of clinical sign and weight loss is shown in Fig. 1. Absolute and percentage of weight decrease with respect to the initial weight at the day of immunization and day 14 after the immunization were 47.7gr and 19.17%, respectively.

Incidence and prevalence of EAE are shown in Fig. 2 and Fig. 3. Five percent of the rats developed the first clinical signs 3 days after immunization. Five percent on day 5, 60% on day 7, 5.26% on day 9, 16.67% on day 10, and 11.11% on day 11 of immunization demonstrated the first clinical signs.

To give a correct imagination of the survival rates, survival analysis was performed, as shown in Fig. 4. Thirty five percent of the animals died during the course of study, lasted for 15 days.

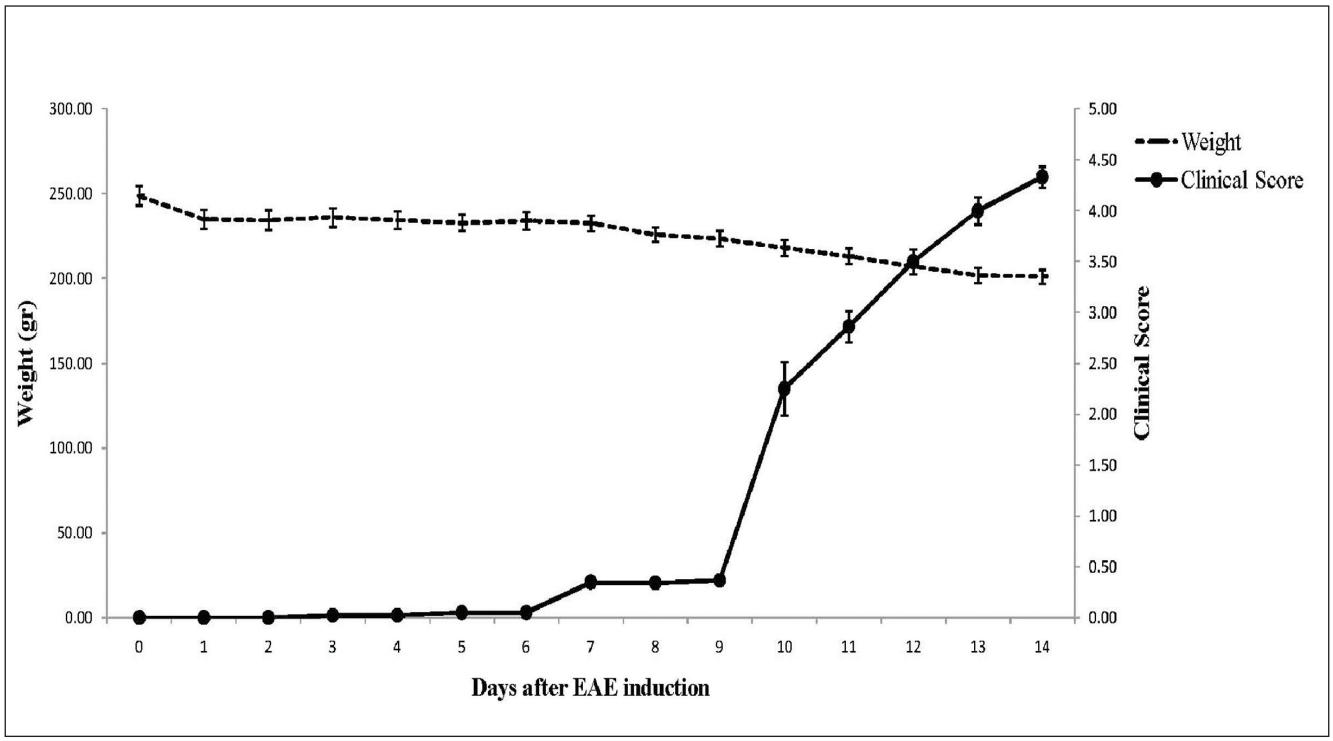

Fig. 1: Course of changes in body weight and clinical scores of male Lewis rats from the day 1 through 14 after the immunization

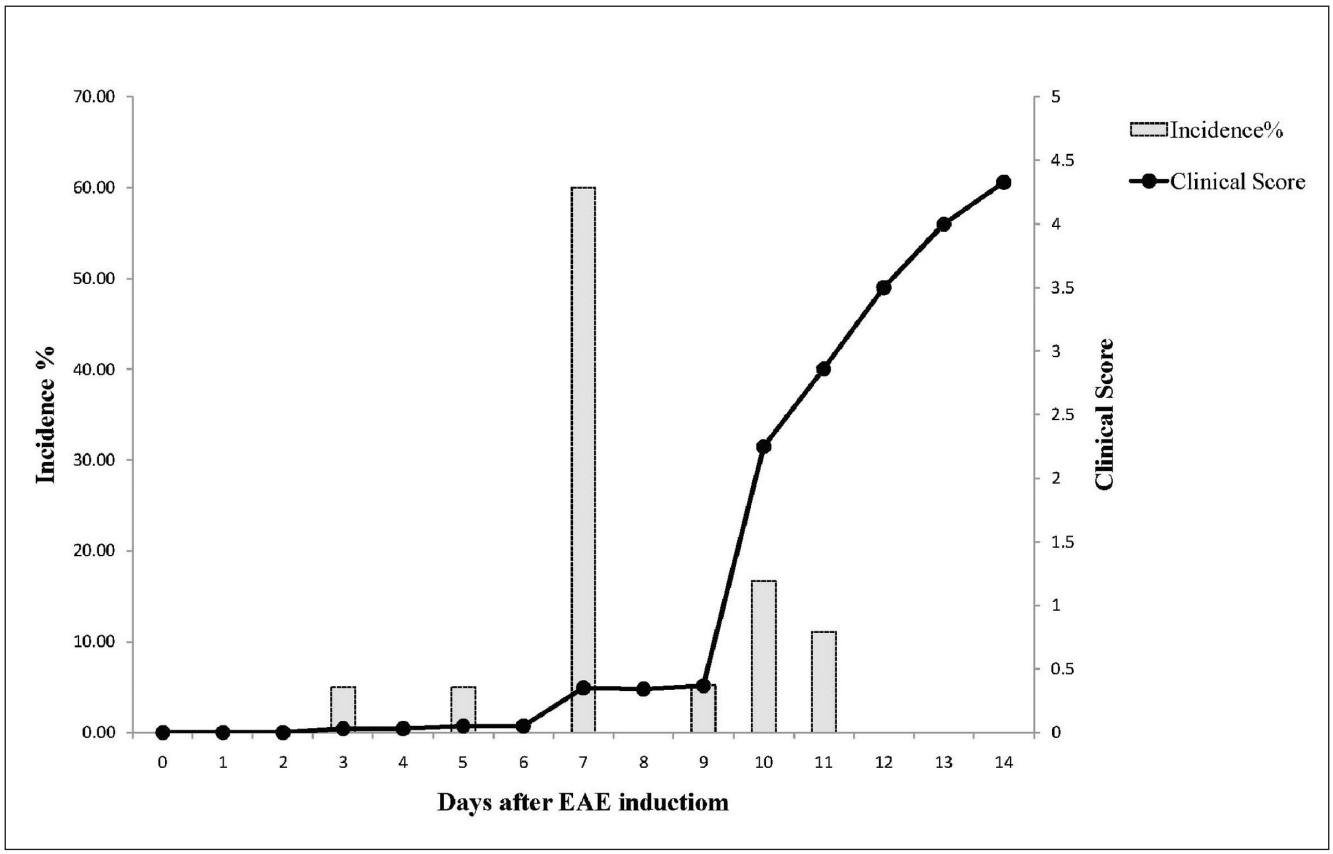

Fig. 2: Incidence of EAE. The incidence was calculated as percentage of the ratio of the number of new diseased rat per number of living rats at each time-point

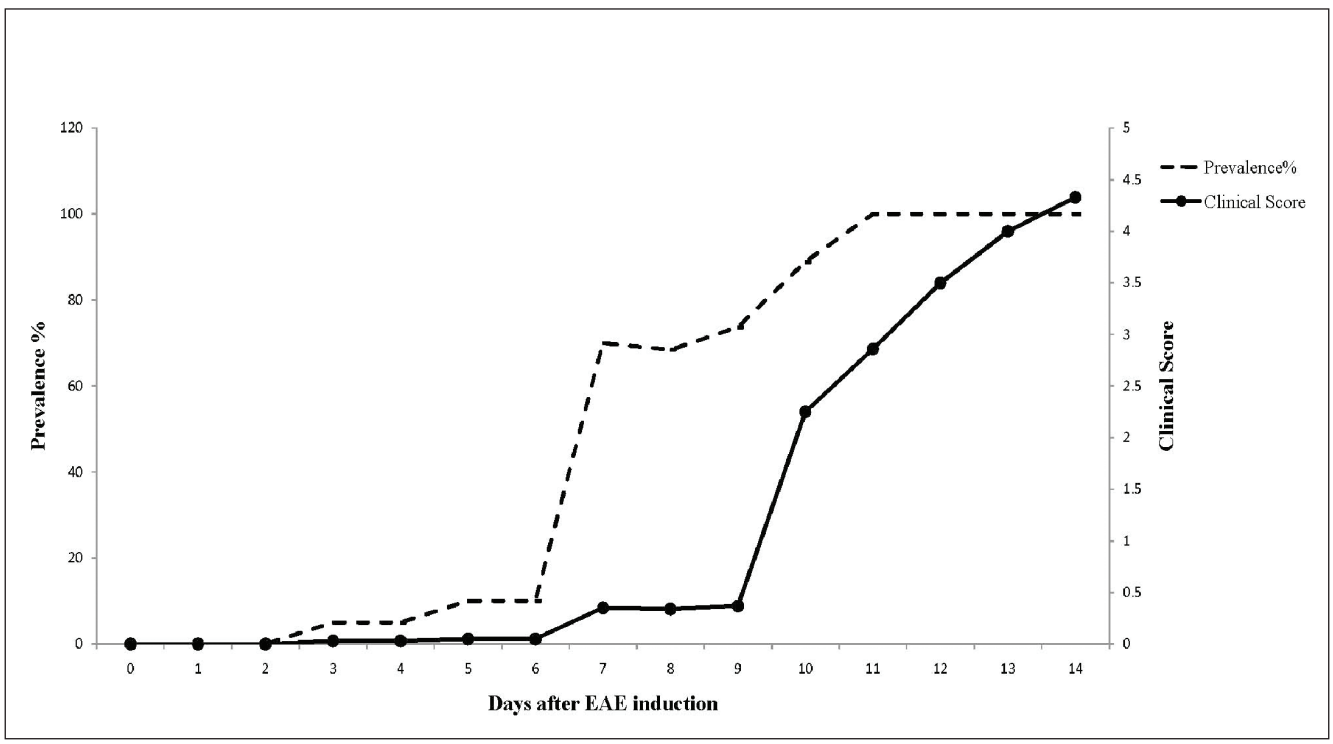

Fig. 3: Prevalence of EAE. The prevalence was calculated as percentage of the ratio of the burden of disease that is new cases plus old cases per number of living rats at each time-point

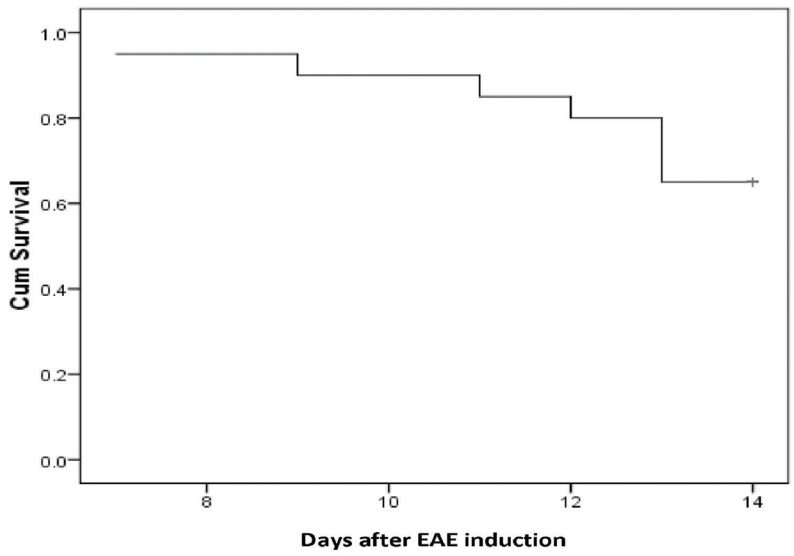

Fig. 4: Survival rate in EAE Lewis rats. Animals were monitored every day for the duration of the experiment

Hepatic inflammation and injury

To examine liver of rats at the peak of the disease, EAE was induced in 5 rats and the liver was dissected. Microscopic view of liver is illustrated in Fig. 5, indicating sinusoidal congestion (Fig. 5a), and there was pycnotic nuclei within hepatocytes (Fig. 5b). Furthermore, hepatocyte necrosis (Fig.5c) and severe widespread congestion was observed. Fat accumulation within hepatocytes (fatty degeneration) (Fig. 5d) was also present.

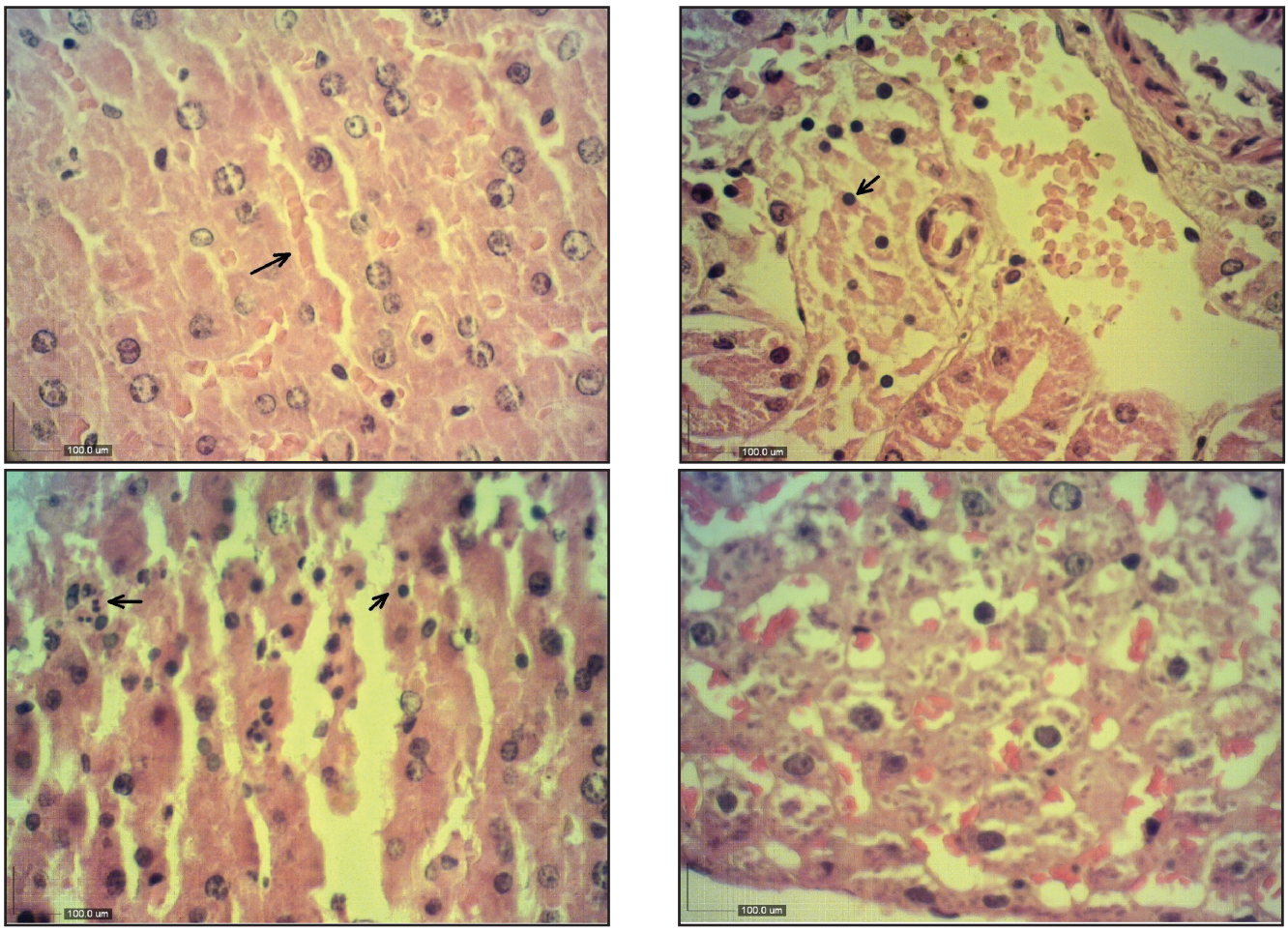

Fig. 5: Light microscopic view of histological analysis of liver lesion of male Lewis rats immunized for EAE at the peak of the clinical course. H&E ×400-stained liver section are characterized by sinusoidal congestion (a), pycnotic nuclei within hepatocytes (b), hepatocyte necrosis and severe widespread congestion (c), as shown by black arrows. Fatty degeneration (d) is also noted as large clear bubbles or vacuoles within the liver cells

Discussion

In this study we demonstrated that the liver of Lewis rats, affected by EAE, have inflammation in their liver. This could be due to migration of extrahepatically activated T cells and/or dysfunction of regulatory T cells (16).

The liver's unique location between the gastrointestinal tract and peripheral lymphoid organs renders this organ the unique ability to induce either tolerance or inflammatory reactions (12, 13). It can also actively modulate ongoing immune reactions by either increased chemotactic attraction of leukocytes (14) or induction of apoptosis of induction of apoptosis of activated intrahepatic lymphocytes (15).

Since the immune response in EAE is towards a self antigen and the self antigen is perpetual, therefore such a prolonged inflammatory response is expected. In addition cytokines such as TNF-α and IFN-γ, as mediators of Th1 response, act as inhibitor of T cell apoptosis (17) and are the interplay between the IFN-γ and counter-regulatory cytokines, such as TGF-β1, the critical cytokine in liver immune response hemostasis (18). They are expected to be involved in development of necroinflammatory liver disease. The inflammatory response in the liver may cause liver injury by hepatocyte apoptosis/necrosis, instead of effector T cell death as shown in this study by pycnotic nuclei within hepatocytes and hepatocyte necrosis. Activation of Liver Sinosoidal Endothelial Cells (LSECs) can be activated in an inflammatory microenvironment and act as competent accessory antigen presenting cells to activate T-cell (18) leading to deletion of LSECs by the activated T cells. It suggests that such an injury to LSECs has an important role in liver injury, because destruction of this sinusoidal cell population abrogates the anatomic barrier allowing unrestricted access of activated T cells to hepatocytes (19, 20). Moreover, as a consequence of LSECs injury, intrasinusoidal thrombosis and tissue hypoxia develop which further worsens liver injury (20). Sinusoidal congestion shown in this study may mention involvement of LSECs in liver injury. Such an inflammatory milieu in the liver (21, 22) may also affect regulatory T cells function (23).

The importance of regulatory T cells and liver effect on suppression of EAE has been previously reported (24) in which EAE was suppressed in transgenic mice expressing myelin in their liver. It follows that switching of liver from a tolerance-inducing organ to an inflammatory state can be involved in exacerbation of the disease. However, direct examination of the liver tissue was not performed in the mentioned study. Our study is the first study which examines liver tissue in animal model of MS and along with the previous report (25) can point to a new complementary diagnostic approach for MS.

Conclusion

Liver has an anti-inflammatory microenvironment. However, liver damage occurs in EAE model of Lewis rat presented in this study. We hypothesize that the important point is the perpetuation of self antigen which leads to continuous migration of extrahepatically activated T cells, abrogation of LSECs, and/or differentiation of liver resident immune cells, as important regulatory cells, towards an inflammatory phenotype. It makes an inflammatory milieu in the liver, which follows migration and development of more inflammatory cells and may and/or paralyses tolerance inducing mechanisms. As the self-antigen exists and activation of T cells occurs, liver injury continues. Considering the important tolerance-inducing role of liver and apart from CNS lesion, liver injury may act as synergistic factor for debilitation and mortality. More research is required to elucidate the underlying mechanisms.

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References

1. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron 2006; 52(1):61-76.

2. Lisak RP, Zweiman B. In vitro cell-mediated immunity of cerebrospinal-fluid lymphocytes to myelin basic protein in primary demyelinating diseases. N Engl J Med 1977; 297(16):850-3.

3. Kurtzke JF, Hyllested K. Multiple sclerosis in the Faroe Islands: I. clinical and epidemiological features. Ann Neurol 1979; 5(1):6-21.

4. Cabre P, Signate A, Olindo S, Merle H, Caparros-Lefebvre D, Béra O, *et al.* Role of return migration in the emergence of multiple sclerosis in the French West Indies. Brain 2005; 128(pt 12):2899-910.

5. Frohman EM, Racke MK, Raine CS. Multiple sclerosis: the plaque and its pathogenesis. N Engl J Med 2006; 354(9):942-55.

6. Carrier P, Godet B, Crepin S, Magy L, Debette-Gratien M, Pillegand B, *et al.* Acute liver toxicity due to methylprednisolone: consider this diagnosis in the context of autoimmunity. Clin Res Hepatol Gastroenterol 2013; 37(1):100-4.

7. Tamura S, Warabi Y, Matsubara S. Severe liver dysfunction possibly caused by the combination of interferon beta-1b therapy and melilot (sweet clover) supplement. J Clin Pharm Ther 2012; 37(6):724-5.

8. Lisotti A, Azzaroli F, Brillanti S, Mazzella G. Severe acute autoimmune hepatitis after natalizumab treatment. Dig Liver Dis 2012; 44(4):356-7.

9. Subramaniam K, Pavli P, Llewellyn H, Chitturi S. Glatiramer acetate induced hepatotoxicity. Curr Drug Saf 2012; 7(2):186-8.

10. Larijani B, Esfahani EN, Amini P, Nikbin B, Alimoghaddam K, Amiri S, *et al.* Stem cell therapy in treatment of different diseases. Acta Med Iran 2012; 50(2):79-96.

11. Prinz-Hadad H, Mizrachi T, Irony-Tur-Sinai M, Prigozhina TB, Aronin A, Brenner T, *et al.* Amelioration of autoimmune neuroinflammation by the fusion molecule Fn14·TRAIL. J Neuroinflammation 2013; 10:36-46.

12. Cantor HM, Dumont AE. Hepatic suppression of sensitization to antigen absorbed into the portal system. Nature 1967; 215(5102):744–5.

13. Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology 2006; 43(2 Suppl 1): S54-62.

14. Bertolino P, Bowen DG, McCaughan GW, Fazekas De St Groth B. Antigen-specific primary activation of CD8(+) T cells within the liver. J Immunol 2001; 166(5):5430–8.

15. Liu ZX, Govindarajan S, Okamoto S, Dennert G. Fas-mediated apoptosis causes elimination of virus-specific cytotoxic T cells in the virus-infected liver. J Immunol 2001; 166(5):3035–3041.

16. Mehal WZ, Juedes AE, Crispe IN. Selective retention of activated CD8+T cells by the normal liver. J Immunol 1999; 163(6): 3202–10.

17. Bradham CA, Plumpe J, Manns MP, Brenner DA, Trautwein C. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. Am J Physiol 1998; 275(3 pt 1):G387–G392.

18. Lin JT, Martin SL, Xia L, Gorham JD. TGF-beta 1 uses distinct mechanisms to inhibit IFN-gamma expression in CD4+T cells at priming and at recall: differential involvement of Stat4 and T-bet. J Immunol 2005; 174(10):5950–8.

19. Knolle PA, Gerken G, Loser E, Dienes HP, Gantner F, Tiegs G, *et al.* Role of sinusoidal endothelial cells of the liver in concanavalin A-induced hepatic injury in mice. Hepatology 1996; 24(2):824–9.

20. Khandoga A, Hanschen M, Kessler JS, Krombach F. CD4+T cells contribute to postischemic liver injury in mice by interacting with sinusoidal endothelium and platelets. Hepatology 2006; 43(2): 306–15.

21. Osman Y, Kawamura T, Naito T, Takeda K, Van Kaer

L, Okumura K, *et al.* Activation of hepatic NKT cells and subsequent liver injury following administration of alpha-galactosylceramide. Eur J Immunol 2000; 30(7):1919–28.

22. Toyabe S, Seki S, Iiai T, Takeda K, Shirai K, Watanabe H, *et al.* Requirement of IL-4 and liver NK1+ T cells for concanavalin A induced hepatic injury in mice. J Immunol 1997; 159(3):1537–42.

23. Longhi MS, Ma Y, Mitry RR, Bogdanos DP, Heneghan M, Cheeseman P, *et al.* .Effect of CD4+CD25+ regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis. J Autoimmun 2005; 25(1):63–71.

24. Lüth S, Huber S, Schramm C, Buch T, Zander S, Stadelmann C, *et al.* Ectopic expression of neural autoantigen in mouse liver suppresses experimental autoimmune neuroinflammation by inducing antigen-specific Tregs. J Clin Invest 2008; 118(10):3403-10.

25. Tremlett H, Seemüller S, Zhao Y, Yoshida EM, Oger JD, Petkau J. Liver test abnormalities in multiple sclerosis: findings from placebo-treated patients. Neurology 2006 10; 67(7):1291-3.