Erythropoietin and autoimmune neuroinflammation: lessons from experimental autoimmune encephalomyelitis and experimental autoimmune neuritis

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Abstract: Erythropoietin (EPO) is known to have numerous biological functions. While its primary function is during haematopoiesis, recent studies have shown that EPO plays important role in cytoprotection, immunomodulation, and anti-apoptosis. These secondary functions of EPO are integral to tissue protection following hypoxic injury, ischemia-reperfusion injury, and spinal cord injury in the central nervous system. This review focuses on experimental evidence documenting the neuroprotective effects of EPO in organ-specific autoimmune nervous system disorders such as experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune neuritis (EAN). In addition, the immunomodulatory role of EPO in the pathogenesis of EAE and EAN animal models of human multiple sclerosis and Guillain-Barré syndrome, respectively, will be discussed.

Key words: Autoimmune diseases, Erythropoietin, Experimental autoimmune neuritis, Encephalomyelitis, Neuroinflammation, Neuroprotection

Introduction

Erythropoietin (EPO) is a glycoprotein produced in the kidneys of adults that potentiates the number of red blood cells by ensuring the survival of erythroid progenitor cells. EPO stimulates the differentiation and proliferation of these cells via binding to the EPO receptor through oxygen-dependent regulation [1-3]. Several studies have shown that

EPO, acting as an immunomodulator, ameliorates various types of inflammation, including organ-specific autoimmune diseases [1], brain ischemia [1, 4, 5], and spinal cord injury [6]. However, EPO treatment is also implicated in the adverse production of unwanted excess erythrocytes [7]. To this end, tissue-protective EPO derivatives (e.g., carbamyl EPO), which have less of an adverse effect on erythropoiesis, have been studied [1] in order to specifically investigate the induction of the non-haematopoietic tissue-protective capabilities of EPO. EPO has also been shown to deleteriously inhibit macrophage function in bacterial-infected animal models [2]. Thus, the role of EPO is unique depending on the type of inflammation in *in vivo* models. Although it has been suggested that EPO has beneficial effects in autoimmune disease models [8-10],

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a cumulative review on the role of EPO in organ-specific autoimmune diseases has not yet been published.

Experimental autoimmune encephalomyelitis (EAE), a model of central nervous system (CNS) demyelinating multiple sclerosis (MS), is a prototype of organ-specific autoimmune diseases. It is characterized by the proliferation of antigen-specific autoimmune T-cells in peripheral immune tissues, the circulation of T-cells in the blood, the homing of autoimmune T-cells with classical M1 macrophages in the target organ (i.e., spinal cords), antigen presentation in the target organ, and the elimination of T-cells via apoptosis [11-14]. During the process of T-cell infiltration, regulatory T-cells and alternatively-activated M2 macrophages also infiltrate the target organ [15]. Because the immunomodulatory roles of regulatory T-cells and M2 macrophages operate in conjunction with the induction of inflammation, these cell types may contribute to the amelioration of inflammation through the secretion of anti-inflammatory mediators. During autoimmune disease in the CNS, neurons and glial cells would be affected by cytokines secreted from inflammatory cells as well as by oxidative stress [16]. Of these cells, some are destroyed while others persist via the expression of cytoprotective enzymes including heat shock proteins (HSP) [17, 18], osteopontin [19], and EPO [20]. Thus, a therapeutic target would be the decrease of pro-inflammatory cytokines and/or the increase of cytoprotective factors that have antioxidant and neuroprotective capacities.

Experimental autoimmune neuritis (EAN), a model of the human autoimmune disorder known as Guillain-Barré syndrome (GBS), is induced by the sensitization of neuritogenic antigens in susceptible animals [21-24]. Following immunization of the neuritogenic antigen, the pathogenesis of EAN is characterized by the proliferation of autoreactive T-cells, the migration of T-cells and bystander macrophages in peripheral nervous system (PNS) tissues, and the induction of PNS paralysis [23, 24]. The pathogenesis of EAN is similar to that of EAE although the target organs in EAN are various tissues in the PNS rather than the CNS.

The aim of the present review was to discuss the role of EPO in the course of the autoimmune disease models of EAE [11] and EAN [25], which are associated with human CNS-demyelinating MS and PNS-demyelinating GBS, respectively.

EPO-Inducible Signal Pathways

The structure and signal networks of EPO and EPO receptors *in vitro* are well summarized in previous review papers [2, 26]. There is general agreement that, under low oxygen conditions, EPO production is initiated in the kidneys through the induction of transcription protein hypoxia inducible factor [2]. EPO, via binding with its receptor, is known to repress nuclear factor-kappa B (NF-kB), an important transcription factor in the production of pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-alpha, and inducible nitric oxide synthase (iNOS). Of course, several other signal pathways are also involved, including mitogen-activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K) [2]. The EPO receptor has been detected in a variety of tissues, including the CNS, the PNS, heart, lungs, kidneys, pancreas, liver, and immune organs, suggesting that EPO may function in all organs through the binding of EPO receptors [26, 27]. Considering that EPO represses NF-kB, a key signal in the production of pro-inflammatory cytokines in EAE [28, 29], it is believed to have an anti-inflammatory role despite the adverse side effect of excess erythropoiesis.

Possible Involvement of EPO in Organ-Specific Autoimmune Neurological Diseases

When considering pathogenic factors in the course of autoimmune diseases, including EAE [12-14] and EAN [21, 22], the blocking and/or suppression of each step is recommended to inhibit progression of the disease. Because the majority of T-cells in target organs are primed in PNS immune systems, it is assumed that T-cells are eliminated through apoptosis in these organs [30]. Thus, treatment strategies for autoimmune diseases generally consist of 1) the suppression of autoimmune T-cells [31, 32], 2) blocking the homing of autoimmune T-cells into the target organs [33], 3) generation and activation of anti-inflammatory factors such as regulatory T-cells and M2 macrophages [15, 34, 35], and 4) neuroprotection of target organ cells, even though the infiltration of some inflammatory cells is possible [12-14, 36]. Thus, the application of EPO, which has both neuroprotective and immunoregulatory capabilities, may act as a beneficial factor in organ-specific neuroinflammation models.

Suppression of Immune Cells by EPO through the Repression of T-Cell Proliferation and Pro-inflammatory Cytokines in the Peripheral Immune Systems

The generation of autoreactive T-cells is believed to play a key role in the pathogenesis of autoimmune diseases, including EAE [12-14] and EAN [21, 22]. It has been found that EPO treatment in mice with EAE [37] and Lewis rats with EAN [10] following the immunization of organ-specific antigens leads to a significant suppression of the proliferation of autoimmune T-cells. In myelin oligodendrocyte glycoprotein (MOG)-induced EAE in mice, EPO treatment has direct immunomodulatory effects within the peripheral lymphoid tissue via the significant reduction of all subsets of inflammatory cells. This includes CD4+ and CD8+ T-cells, CD11c+ dendritic cells, and major histocompatibility complex (MHC) class I and class II cell expression in the draining inguinal lymph node cells [37]. A decreased number of mononuclear cells in peripheral lymphoid tissues is associated with a decreased production of pro-inflammatory cytokines such as IL-6, TNF-alpha, IL-2, and interferon (IFN)-gamma either from T-cells and/or macrophages. Conversely, EPO treatment induces the expansion of peripheral regulatory T-cells in MOG-EAE mice, which counteracts the action of pro-inflammatory encephalitogenic T-cells. The suppressive effect of EPO in pro-inflammatory cytokine production (either TNF-alpha, or IFN-gamma, IL-1 beta, IL-17, etc.) has also been identified in the spinal cord and peripheral lymphoid tissues in mouse EAE models [38, 39]. Furthermore, EPO reduces the expression of MHC-II cells on peripheral antigen presentation cells [39]. Together, these findings suggest that EPO directly interrupts two important steps for the induction and progression of autoimmune diseases in peripheral immune systems; T-cell proliferation and antigen presentation.

The increased level of EPO in EAN rat tissues has been studied using immunohistochemical techniques, suggesting that EPO is endogenously produced in EAN tissues [36]. As for the role of EPO in T-cell proliferation, *in vitro* experiments has revealed that proliferation of T-cells was reduced in the presence of EPO [10]. EPO treatment in an *in vivo* rat EAN model demonstrated the induction of anti-inflammatory cytokine transforming growth factor (TGF)-beta [10], which agrees with the findings of studies investigating EPO in EAE. Thus, it is highly possible that EPO treatment in EAN, either endogenously or exogenously, may influence the suppression of T-cell proliferation and subsequent decreases of pro-inflammatory cytokines.

The possibility that EPO suppresses inflammatory cells in target organs, in both CNS and PNS tissues, cannot be excluded. However, EPO treatment in EAE and EAN animal models may preferentially repress not only pro-inflammatory cytokines from T-cells and/or macrophages, but may also induce anti-inflammatory TGF-beta in peripheral immune systems. Thus, EPO treatment in autoimmune disease models is likely to be associated with suppression of the proliferation of autoimmune T-cells, and antigen presentation in peripheral immune tissues with multiple signal pathways, but is less relevant to CNS and PNS target organs, if at all. This may contribute to the amelioration of autoimmune inflammation, irrespective of the target organ.

Induction of Antioxidant Proteins by EPO Treatment in Brain Inflammation

EPO is known to induce neural heme oxygenase-1 (HO-1), also known as HSP-32, expression through the activation of PI3K, MAPK, and nuclear factor erythroid 2-related factor 2 (Nrf2) pathways *in vitro* [40]. Of these, HO-1 is regarded as an antioxidant stress protein that contributes to tissue protection [41-43]. Oxidative stress is associated with deterioration in autoimmune diseases [16, 44]. An immunohistochemical study found that HSP-27 plays an important role in recovery from EAE via tissue protection [18]. Following treatment with EPO in mouse models of EAE, there was a significantly higher expression of HO-1 in the CNS and spleen in conjunction with a decreased expression of pro-inflammatory cytokines, including IFN-gamma, but an increased expression of IL-4 and IL-10 [8, 45]. Nrf2, a nuclear factor involved in EPO signaling [40], has also been identified as an important antioxidant that is cytoprotective following head injury experiments in mice [46] and early brain injury subsequent to subarachnoid hemorrhage in rats [47]. Thus, it is postulated that EPO induces antioxidant activity through the upregulation of HO-1 and Nrf2, and suppresses levels of pro-inflammatory cytokines. These actions probably contribute to the modulation of immune cells in the CNS and PNS that result in the protection of neurons in EAE models [48, 49].

Protection of Neurons by EPO in Neuroinflammation

EPO is produced in a variety of cell types, including neurons and glial cells, of normal nervous tissues [50, 51]. Following CNS injury, EPO is stimulated with hypoxia-inducible factors, and has been found to be activated and to protect neurons after ischemia-reperfusion injury in animal models [5, 52]. With the immunization of organ-specific antigens, semi-quantitative analysis has demonstrated that the expression of EPO is upregulated in the CNS [20] and in the PNS [36], which may contribute to the protection of neurons either by paracrine or autocrine mechanisms. Furthermore, EPO has been immune-detected in neurons, glial cells, and macrophages in EAE [20] and EAN [36] models. In EAE models, there is no doubt that neurons and glial cells exhibit an increased immunoreactivity of EPO, but the influence of EPO on macrophages requires further analysis. In these models, the co-localization of EPO with either IFN-gamma or TNF-alpha reciprocally inhibits their production in neurons and glial cells [35], and this suppression may contribute to the neuroprotective capabilities of EPO. The involvement of EPO with macrophages in EAE remains under debate.

There are two different phenotypes of macrophages: classically-activated M1 macrophages and alternatively-activated M2 macrophages [53, 54]. In the case of spontaneous recovery from paralysis in rat EAE models, there is a predominance of M2 macrophages expressing arginase-1, which is a marker for the M2 phenotype [55]. Regarding its role in macrophages, EPO suppresses pro-inflammatory iNOS [20, 55] and negatively activates macrophages, which express TGF-beta [10]. This indicates that EPO plays an anti-inflammatory role in extra–erythropoietic organs [2], just as it does in EAE animals models.

Conclusion

EPO, a glycoprotein produced by the kidneys in adults, plays an important role, not only in haematopoiesis but also in immunomodulation and tissue protection. This review summarizes the potential role of EPO in organ-specific neuroinflammation models such as EAE and EAN. Based on the immunomodulatory effects and the neuroprotective capabilities of EPO, it is presented as a good candidate for the therapeutic regulation of autoimmune diseases at the induction stage. However, the adverse side effects of EPO, including excess erythropoiesis, need to be further examined by future studies so that they may be overcome.

Acknowledgements

References

1. Brines M, Cerami A. Erythropoietin-mediated tissue protection: reducing collateral damage from the primary injury response. J Intern Med 2008;264:405-32.
2. Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G. The pleiotropic effects of erythropoietin in infection and inflammation. Microbes Infect 2012;14:238-46.
3. Sasaki R, Masuda S, Nagao M. Erythropoietin: multiple physiological functions and regulation of biosynthesis. Biosci Biotechnol Biochem 2000;64:1775-93.
4. Velly L, Pellegrini L, Guillet B, Bruder N, Pisano P. Erythropoietin 2nd cerebral protection after acute injuries: a double-edged sword? Pharmacol Ther 2010;128:445-59.
5. van der Kooij MA, Groenendaal F, Kavelaars A, Heijnen CJ, van Bel F. Neuroprotective properties and mechanisms of erythropoietin in *in vitro* and *in vivo* experimental models for hypoxia/ischemia. Brain Res Rev 2008;59:22-33.
6. Matis GK, Birbilis TA. Erythropoietin in spinal cord injury. Eur Spine J 2009;18:314-23.
7. Patel NS, Nandra KK, Thiemermann C. Bench-to-bedside review: Erythropoietin and its derivatives as therapies in critical care. Crit Care 2012;16:229.
8. Chen SJ, Wang YL, Lo WT, Wu CC, Hsieh CW, Huang CF, Lan YH, Wang CC, Chang DM, Sytwu HK. Erythropoietin enhances endogenous haem oxygenase-1 and represses immune responses to ameliorate experimental autoimmune encephalomyelitis. Clin Exp Immunol 2010;162:210-23.
9. Agnello D, Bigini P, Villa P, Mennini T, Cerami A, Brines ML, Ghezzi P. Erythropoietin exerts an anti-inflammatory effect on the CNS in a model of experimental autoimmune encephalomyelitis. Brain Res 2002;952:128-34.
10. Mausberg AK, Meyer Zu Hörste G, Dehmel T, Stettner M, Lehmann HC, Sheikh KA, Kieseier BC. Erythropoietin ameliorates rat experimental autoimmune neuritis by inducing transforming growth factor-beta in macrophages. PLoS One 2011;6:e26280.
11. Shin T, Ahn M, Matsumoto Y. Mechanism of experimental autoimmune encephalomyelitis in Lewis rats: recent insights from macrophages. Anat Cell Biol 2012;45:141-8.
12. Wekerle H. Lessons from multiple sclerosis: models, concepts, observations. Ann Rheum Dis 2008;67 Suppl 3:iii56-60.
13. Kapadia M, Sakic B. Autoimmune and inflammatory mechanisms of CNS damage. Prog Neurobiol 2011;95:301-33.

14. Pierson E, Simmons SB, Castelli L, Goverman JM. Mechanisms regulating regional localization of inflammation during CNS autoimmunity. Immunol Rev 2012;248:205-15.

15. Mikita J, Dubourdieu-Cassagno N, Deloire MS, Vekris A, Biran M, Raffard G, Brochet B, Canron MH, Franconi JM, Boiziau C, Petry KG. Altered M1/M2 activation patterns of monocytes in severe relapsing experimental rat model of multiple sclerosis. Amelioration of clinical status by M2 activated monocyte administration. Mult Scler 2011;17:2-15.

16. Herz J, Zipp F, Siffrin V. Neurodegeneration in autoimmune CNS inflammation. Exp Neurol 2010;225:9-17.

17. Mansilla MJ, Montalban X, Espejo C. Heat shock protein 70: roles in multiple sclerosis. Mol Med 2012;18:1018-28.

18. Kim H, Moon C, Ahn M, Byun J, Lee Y, Kim MD, Matsumoto Y, Koh CS, Shin T. Heat shock protein 27 upregulation and phosphorylation in rat experimental autoimmune encephalomyelitis. Brain Res 2009;1304:155-63.

19. Shin T. Osteopontin as a two-sided mediator in acute neuroinflammation in rat models. Acta Histochem 2012;114:749-54.

20. Kang SY, Kang JH, Choi JC, Lee JS, Lee CS, Shin T. Expression of erythropoietin in the spinal cord of lewis rats with experimental autoimmune encephalomyelitis. J Clin Neurol 2009;5:39-45.

21. Lu MO, Zhu J. The role of cytokines in Guillain-Barré syndrome. J Neurol 2011;258:533-48.

22. Hahn AF. Experimental allergic neuritis (EAN) as a model for the immune-mediated demyelinating neuropathies. Rev Neurol (Paris) 1996;152:328-32.

23. Ahn M, Moon C, Lee Y, Koh CS, Kohyama K, Tanuma N, Matsumoto Y, Kim HM, Kim SR, Shin T. Activation of extracellular signal-regulated kinases in the sciatic nerves of rats with experimental autoimmune neuritis. Neurosci Lett 2004;372:57-61.

24. Lee Y, Shin T. Expression of constitutive endothelial and inducible nitric oxide synthase in the sciatic nerve of Lewis rats with experimental autoimmune neuritis. J Neuroimmunol 2002;126:78-85.

25. Busch SA, Hamilton JA, Horn KP, Cuascut FX, Cutrone R, Lehman N, Deans RJ, Ting AE, Mays RW, Silver J. Multipotent adult progenitor cells prevent macrophage-mediated axonal dieback and promote regrowth after spinal cord injury. J Neurosci 2011;31:944-53.

26. Farrell F, Lee A. The erythropoietin receptor and its expression in tumor cells and other tissues. Oncologist 2004;9 Suppl 5:18-30.

27. Jelkmann W, Bohlius J, Hallek M, Sytkowski AJ. The erythropoietin receptor in normal and cancer tissues. Crit Rev Oncol Hematol 2008;67:39-61.

28. Ellrichmann G, Thöne J, Lee DH, Rupec RA, Gold R, Linker RA. Constitutive activity of NF-kappa B in myeloid cells drives pathogenicity of monocytes and macrophages during autoimmune neuroinflammation. J Neuroinflammation 2012; 9:15.

29. Hwang I, Ha D, Ahn G, Park E, Joo H, Jee Y. Experimental autoimmune encephalomyelitis: association with mutual regulation of RelA (p65)/NF-kappaB and phospho-IkappaB in

the CNS. Biochem Biophys Res Commun 2011;411:464-70.

30. Moon C, Kim S, Wie M, Kim H, Cheong J, Park J, Jee Y, Tanuma N, Matsumoto Y, Shin T. Increased expression of p53 and Bax in the spinal cords of rats with experimental autoimmune encephalomyelitis. Neurosci Lett 2000;289:41-4.

31. Wang S, Yan C, Xu H, Zhao X, Han Y. Suppression of encephalitogenic T-cell responses by cilostazol is associated with upregulation of regulatory T-cells. Neuroreport 2010;21:629-35.

32. Ishii H, Jin X, Ueno M, Tanabe S, Kubo T, Serada S, Naka T, Yamashita T. Adoptive transfer of Th1-conditioned lymphocytes promotes axonal remodeling and functional recovery after spinal cord injury. Cell Death Dis 2012;3:e363.

33. Sheremata WA, Minagar A, Alexander JS, Vollmer T. The role of alpha-4 integrin in the aetiology of multiple sclerosis: current knowledge and therapeutic implications. CNS Drugs 2005;19:909-22.

34. Liu Y, Teige I, Birnir B, Issazadeh-Navikas S. Neuron-mediated generation of regulatory T-cells from encephalitogenic T-cells suppresses EAE. Nat Med 2006;12:518-25.

35. Mengozzi M, Cervellini I, Bigini P, Martone S, Biondi A, Pedotti R, Gallo B, Barbera S, Mennini T, Boraso M, Marinovich M, Petit E, Bernaudin M, Bianchi R, Viviani B, Ghezzi P. Endogenous erythropoietin as part of the cytokine network in the pathogenesis of experimental autoimmune encephalomyelitis. Mol Med 2008;14:682-8.

36. Ahn M, Moon C, Jeong C, Matsumoto Y, Koh CS, Shin T. Upregulation of erythropoietin in rat peripheral nervous system with experimental autoimmune neuritis. Brain Res 2010;1333:82-90.

37. Yuan R, Maeda Y, Li W, Lu W, Cook S, Dowling P. Erythropoietin: a potent inducer of peripheral immuno/inflammatory modulation in autoimmune EAE. PLoS One 2008;3:e1924.

38. Savino C, Pedotti R, Baggi F, Ubiali F, Gallo B, Nava S, Bigini P, Barbera S, Fumagalli E, Mennini T, Vezzani A, Rizzi M, Coleman T, Cerami A, Brines M, Ghezzi P, Bianchi R. Delayed administration of erythropoietin and its non-erythropoietic derivatives ameliorates chronic murine autoimmune encephalomyelitis. J Neuroimmunol 2006;172:27-37.

39. Zhang X, Li QY, Xiao BG. Anti-inflammatory effect of erythropoietin therapy on experimental autoimmune encephalomyelitis. Int J Neurosci 2012;122:255-62.

40. Genc K, Egrilmez MY, Genc S. Erythropoietin induces nuclear translocation of Nrf2 and heme oxygenase-1 expression in SH-SY5Y cells. Cell Biochem Funct 2010;28:197-201.

41. Kalmar B, Greensmith L. Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 2009;61:310-8.

42. Blancou P, Tardif V, Simon T, Rémy S, Carreño L, Kalergis A, Anegon I. Immunoregulatory properties of heme oxygenase-1. Methods Mol Biol 2011;677:247-68.

43. Wunder C, Potter RF. The heme oxygenase system: its role in liver inflammation. Curr Drug Targets Cardiovasc Haematol Disord 2003;3:199-208.

44. Diem R, Sättler MB, Bähr M. Neurodegeneration and -protec-

tion in autoimmune CNS inflammation. J Neuroimmunol 2007;184:27-36.

45. Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J Neurol 2004;251:261-8.

46. Jin W, Kong J, Lu T, Wang H, Ni H, Wu J, Dai Y, Jiang J, Liang W. Erythropoietin prevents secondary brain injury induced by cortical lesion in mice: possible involvement of Nrf2 signaling pathway. Ann Clin Lab Sci 2011;41:25-32.

47. Zhang J, Zhu Y, Zhou D, Wang Z, Chen G. Recombinant human erythropoietin (rhEPO) alleviates early brain injury following subarachnoid hemorrhage in rats: possible involvement of Nrf2-ARE pathway. Cytokine 2010;52:252-7.

48. Jazwa A, Cuadrado A. Targeting heme oxygenase-1 for neuro-protection and neuroinflammation in neurodegenerative dise-ases. Curr Drug Targets 2010;11:1517-31.

49. Cuadrado A, Rojo AI. Heme oxygenase-1 as a therapeutic target in neurodegenerative diseases and brain infections. Curr Pharm Des 2008;14:429-42.

50. Buemi M, Cavallaro E, Floccari F, Sturiale A, Aloisi C, Trimarchi M, Grasso G, Corica F, Frisina N. Erythropoietin and the brain: from neurodevelopment to neuroprotection. Clin Sci (Lond) 2002;103:275-82.

51. Genc S, Koroglu TF, Genc K. Erythropoietin and the nervous system. Brain Res 2004;1000:19-31.

52. Minnerup J, Heidrich J, Rogalewski A, Schäbitz WR, Wellmann J. The efficacy of erythropoietin and its analogues in animal stroke models: a meta-analysis. Stroke 2009;40:3113-20.

53. Gong D, Shi W, Yi SJ, Chen H, Groffen J, Heisterkamp N. TGFbeta signaling plays a critical role in promoting alternative macrophage activation. BMC Immunol 2012;13:31.

54. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front Biosci 2008;13:453-61.

55. Ahn M, Yang W, Kim H, Jin JK, Moon C, Shin T. Immuno-histochemical study of arginase-1 in the spinal cords of Lewis rats with experimental autoimmune encephalomyelitis. Brain Res 2012;1453:77-86.