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MS-275, AN HISTONE DEACETYLASE INHIBITOR, REDUCES THE INFLAMMATORY REACTION IN RAT EXPERIMENTAL AUTOIMMUNE NEURITIS

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Abstract—Experimental autoimmune neuritis (EAN) is a T cell-mediated autoimmune inflammatory demyelinating disease of the peripheral nervous system and serves as the animal model of human inflammatory demyelinating polyradiculoneuropathies. MS-275, a potent histone deacetylase inhibitor currently undergoing clinical investigations for various malignancies, has been reported to demonstrate promising anti-inflammatory activities. In our present study, MS-275 administration (3.5 mg/kg i.p.) to EAN rats once daily from the appearance of first neurological signs greatly reduced the severity and duration of EAN and attenuated local accumulation of macrophages, T cells and B cells, and demyelination of sciatic nerves. Further, significant reduction of mRNA levels of pro-inflammatory interleukin-1β, interferon-γ, interleukine-17, inducible nitric oxide synthase and matrix metalloproteinase-9 was observed in sciatic nerves of MS-275 treated EAN rats. In lymph nodes, MS-275 depressed pro-inflammatory cytokines as well, but increased expression of anti-inflammatory cytokine interleukine-10 and of foxhead box protein3 (Foxp3), a unique transcription factor of regulatory T cells. In addition, MS-275 treatment increased proportion of infiltrated Foxp3$^+$ cells and anti-inflammatory M2 macrophages in sciatic nerves of EAN rats. In summary, our data demonstrated that MS-275 could effectively suppress inflammation in EAN, through suppressing inflammatory T cells, macrophages and cytokines, and inducing anti-inflammatory immune cells and molecules, suggesting MS-275 as a potent candidate for treatment of autoimmune neuropathies.

Key words: experimental autoimmune neuritis, HDAC inhibitor, MS-275, Foxp3$^+$ T cell, M2 macrophage.

Experimental autoimmune neuritis (EAN) is a T cell-mediated autoimmune inflammatory demyelinating disease of the peripheral nervous system (PNS) that mirrors many clinical and immunological features of human acute and chronic inflammatory demyelinating polyradiculoneuropathies, and is widely applied to investigate therapeutic principles and disease mechanisms of inflammatory polyneuropathies (Gold et al., 2000). Existing treatments of inflammatory polyneuropathies can be divided into supportive management, such as good intensive care and respiratory assistance, as well as active treatment including plasma exchange (PEX) and intravenous immunoglobulin (IVIG) (Meyer zu Hörste et al., 2007). Nevertheless, not all polyneuropathies patients respond to PEX or IVIG (Kieseier et al., 2004), and even in those who recover well, residual weakness and loss of motor units can usually be detected and could explain the fatigue that is a common problem (Meyer zu Hörste et al., 2007). Therefore, more efficacious therapeutic options represent an unmet medical need for polyneuropathies.

EAN is characterized by breakdown of the blood-nerve barrier (BNB), robust accumulation of reactive T cells and macrophages in the PNS and demyelination of peripheral nerves. EAN is considered to be a disease dominated by a pathological cellular immune system, in particular by T helper (Th)1 cells and Th1 cytokines, like interferon-γ (IFN-γ), Iinterlukine-1β (IL-1β), are important for the pathogenesis of EAN (Kieseier et al., 2004; Zhu et al., 1998). In addition, the accumulation of T cells, expressing foxhead box protein3 (Foxp3), was found to be associated with disease recovery, indicating a protective role of Foxp3$^+$ cells in EAN (Zhang et al., 2009a).

Macrophages represent the major cell population in the inflamed PNS, serving as antigen-presenting cells and major effector cells of demyelination and are therefore responsible for most of the neuropathological effects (Maurer et al., 2002). Recent studies suggest that a switch of macrophage phenotype from classical activation (M1) to alternative activation (M2) could change the functions of macrophages from inflammatory to anti-inflammatory and tissue repair and thus might favor the outcome of EAN (Mosser and Edwards, 2008). In the PNS, reactive T cells, macrophages and B cells orchestrate a robust local inflammation that causes demyelination and axon degeneration. Therefore, therapeutic compounds that can inhibit PNS inflammation would improve EAN outcome.

MS-275 is a novel benzamide histone deacetylase inhibitor (HDACI) and known to directly inhibit histone deacetylase (HDAC) activity and cause a hyperacetylation of histones (Hess-Stumpp et al., 2007). Chromatin structure remodeling via changes in the levels of histone acetylation is a key mechanism underlying the regulation of gene expression. HDACIs can modulate the ac-

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Abbreviations: BNB, blood nerve barrier; EAE, experimental autoimmune encephalomyelitis; EAN, experimental autoimmune neuritis; Foxp3, fox head box protein3; HDAC, histone deacetylase; HDACI, histone deacetylase inhibitor; IFN-γ, interferon γ; IL, interleukin; iNOS, inducible nitric oxide synthase; IR, immunoreactivity; IVIG, intravenous immunoglobulin; LFB, luxol fast blue; MMP, matrix metallopeptidase; PBS, phosphate buffered saline; PEX, plasma exchange; PNS, peripheral nervous system; TGF-β, transforming growth factor beta; T_h cell, T helper cell; Treg cell, regulatory T cell; VPA, valproic acid.

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tivities of HDAC, and hence activate and/or repress subsets of genes (Lin et al., 2006). Furthermore, recent studies show that certain transcription factors, such as NF-kappaB, can be directly acetylated and thus be influenced by HDACIs as well (Li et al., 2008). HDACIs were first noticed by their antineoplastic activities and several HDACIs are current undergoing clinical investigation for various solid and hematological malignancies (Drummond et al., 2005). Besides anticancer activities, HDACIs recently emerged as potent anti-inflammatory compounds and are effective in several inflammatory models, like experimental autoimmune encephalomyelitis (EAE), asthma and allergic diseases (Zhang et al., 2008a). It was reported that HDACIs could effectively reduce expression of pro-inflammatory cytokines, like IL-1β, IFN-γ, TNF-α, IL-8 and transforming growth factor beta (TGF-β), down-regulate immune stimulators (like IL-6) and inhibit the production of nitric oxide, which all contribute to various inflammatory diseases. It is established that T cell differentiation and a variety of related cytokine expression accompany histone acetylation pattern remodelling (Sawalha, 2008) and HDACIs can change the histone acetylation pattern of a variety of genes to shift the ratios of different Th cell subpopulations (Su et al., 2008).

MS-275 is specific for class I HDACs and preferentially inhibits HDAC1 versus HDAC3 and has no inhibitory activity towards HDAC8 (Blanchard and Chipoy, 2005). Anti-inflammatory potential and activities of MS-275 were recently demonstrated *in vivo* and *in vitro*, respectively. In an animal model of rheumatoid arthritis, MS-275 effectively ameliorated collagen induced arthritis, strongly attenuated paw swelling, bone erosion and resorption, and decreased serum IL-6 and IL-1β levels (Lin et al., 2007). *In vitro*, MS-275 exhibited strong immunomodulatory activities on dendritic cells (Nencioni et al., 2007). As an anti-cancer agent, many promising properties of MS-275 were proven, including good oral bioavailability, linear pharmacokinetics and good tolerance (Ryan et al., 2005). Thus, the proven reliability and safety of MS-275 suggest that it may present an effective treatment of various neurological conditions. Our aim here was to study the anti-inflammatory effects of MS-275 in EAN.

EXPERIMENTAL PROCEDURES

Animals

Male Lewis rats (8–10 weeks, 170–200 g, Charles River, Sulzfeld, Germany) were housed under a 12-h light/dark cycle with free access to food and water. All animal procedures were in accordance with a protocol approved by the local Administration District Official Committee. All efforts were made to minimize the number of animals and their suffering.

EAN induction and MS-275 treatment

EAN was induced as described (Zhang et al., 2008a). Briefly, rats were immunized by s.c. injection at the basal part of tails with 100 μL of an inoculum containing 100 μg of synthetic neuritogenic P2 peptide 57–81 (GeneScript Corporation, Scotch Plains, NJ, USA). Neurological scores of EAN were evaluated every day as follows: 0=normal, 1=reduced tonus of tail, 2=limp tail, impaired righting, 3=absent righting, 4=gait ataxia, 5=mild paresis of the hind limbs, 6=moderate paraparesis, 7=severe paraparesis or paraplegia of the hind limbs, 8=tetraparesis, 9=moribund, and 10=death.

For therapeutic treatment, EAN rats received i.p. injection of MS-275 (Alexis Biochemicals, Loerrach, Germany) (3.5 mg/kg) daily from day 10 to day 14 (six rats/group). For injection, MS-275 was suspended in phosphate buffered saline (PBS) and the same volume (1 ml) of PBS was given to control rats.

We performed therapeutic treatment of EAN three times, six rats per group were included in a 21-days experiment for scoring of neurological signs and body weight; also six rats per group were included in a 15-days EAN experiment to provide material for immunohistochemical analysis. Tissue for PCR analysis was taken from additional groups of rats (n=3) of 15-days EAN experiments.

Immunohistochemistry

To evaluate inflammatory cell infiltration and pathological changes in the PNS, six MS-275 treated or control EAN rats from day 15 were sacrificed. Both sciatic nerves were taken from each rat, processed and further immunohistochemical staining was carried out as described previously (Zhang et al., 2008a). For immunohistochemistry, sciatic nerves were cut to two equal long segments so four cross-sections were analysed for each rat. Following antibodies were used in our present study: CD3 (1:50; Serotec, Oxford, UK) for T-lymphocytes, OX22 (1:200; Serotec, Oxford, UK) for B cells, ED1 for activated macrophages or microglia (1:100; Serotec, Oxford, UK), ED2 for anti-inflammatory macrophages (1:100; Serotec, Oxford, UK), IL-17 (1:100; Santa Cruz, Heidelberg, Germany) and Foxp3 (1:250; eBioscience, Frankfurt, Germany) for distinct T cell populations.

To evaluate immunostaining data, the percentages of areas of immunoreactivity (IR) to areas of sciatic nerve cross-sections were calculated. Briefly, images of sciatic nerve cross-sections were captured under 50× magnification using Nikon Coolscope (Nikon, Düsseldorf, Germany) with fixed parameters. Images were analysed using MetaMorph Offline 7.1 (Molecular Devices, Toronto, Canada). Areas of IR were selected by color threshold segmentation and all parameters were fixed for all images. Areas of sciatic nerve cross-sections were manually selected and were measured using software MetaMorph Offline 7.1. For each EAN rat, four cross-sections from root and middle levels of both sides were analyzed. Results were given as arithmetic means of percentages of areas of IR to areas of sciatic nerve cross-sections and standard errors of means (SEM). Additionally, to investigate accumulation of $Foxp3^+$ and $ED2^+$ cells, the proportions $Foxp3^+$ cells among T cells and $ED2^+$ among macrophages of sciatic nerve cross-sections were calculated. The density of $Foxp3^+$ and $CD3^+$ cells in sciatic nerves was manually counted under 100× magnification. Results were given as arithmetic means of percentages of $Foxp3^+$ cell numbers compared to $CD3^+$ cell number, and ED2 IR areas compared to ED1 IR areas of sciatic nerve cross-sections, and SEM as well.

The routine Luxol Fast Blue (LFB) staining was applied to show myelin. Histological changes between MS-275 and control EAN rats were compared by an established semi-quantitative method. Briefly, four cross-sections from root and middle level of both sides of EAN rats were analyzed. All perivascular areas present in cross-sections were evaluated by two observers unaware of treatment, and the degree of pathological alteration was graded semiquantitatively on the scale described previously (Zhang et al., 2008a). Results were given as mean histological score.

Tissue preparation, RNA isolation, reverse transcription and real-time semi-quantitative PCR

Sciatic nerves and inguinal lymph nodes were quickly removed from EAN rats after intracardial perfusion with 4 °C PBS and stored in liquid nitrogen. Total RNA was isolated using Trizol LS