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Cutting Edge: Requirement for Growth Hormone-Releasing Hormone in the Development of Experimental Autoimmune Encephalomyelitis

Hideto Ikushima,1 Masaharu Kanaoka, and Shinichi Kojima

Growth hormone (GH)-releasing hormone (GHRH) is a neuropeptide that stimulates secretion of GH from the pituitary gland. Although GHRH and its receptor (GHRHR) are expressed in leukocytes, physiological function of GHRH in the immune system remains unclear. To study the influence of GHRH in autoimmunity, susceptibility to experimental autoimmune encephalomyelitis (EAE) was examined in C57BL/6J-Ghrhrlit/lit (lit/lit), mice deficient in the GHRHR gene. We found that lit/lit mice were resistant to myelin oligodendrocyte glycoprotein (MOG)-induced EAE. Splenocytes from MOG-immunized lit/lit mice proliferated normally in response to MOG peptide, suggesting that activation of MOG-specific T cells in GHRHR-deficient mice is not impaired. Our data strongly suggest that GHRH plays a crucial role in the development of EAE and may provide the basis for a novel therapeutic approach protecting from autoimmune diseases. The Journal of Immunology, 2003, 171: 2769–2772.

In this study, we investigated the contribution of GHRH to the pathogenesis of EAE. We report that upon immunization with immunodominant MOG35–55 peptide, GHRHR-deficient lit/lit mice did not develop any neurological impairment. Splenocytes from MOG-immunized lit/lit mice proliferated normally in response to MOG peptide, whereas anti-MOG IgG was increased in lit/lit mice. Our data presented here suggest that GHRH is playing a critical role in the development of EAE in mice and raise a possibility that the GHRH antagonist is beneficial in the treatment of MS.

Materials and Methods

Animal experimentation

Animal experiments in this study were performed according to the guidelines of the Animal Studies Committee of the Research Division of Sumitomo Pharmaceuticals. GHRHR-deficient C57BL/6J-Ghrhrlit/lit mice (lit/lit), their heterozygous littermates (lit/+), control C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were female, 6 wk of age at the time of immunization, and were bred at the Animal Facility (Research Division, Sumitomo Pharmaceuticals) under pathogen-free conditions.

Ag and reagents

The immunodominant MOG35–55 peptide (MEGVYRSPFSRVHLYRNGK) was synthesized and purified through HPLC by Qiagen (Tokyo, Japan). Pertussis toxin was purchased from List Biological Laboratories (Campbell, CA).
Induction and evaluation of EAE

The lit/lit, lit/+ , and control C57BL/6J mice were immunized s.c. with 100 μl of an emulsion composed of MOG35–55, peptide (200 μg/mouse) in saline and an equal volume of CFA containing Mycobacterium tuberculosis H37Ra (Difco, Detroit, MI). Mice received 200 ng of pertussis toxin (List Biological Laboratories) in 0.1 ml of saline (i.p.) on the day of and 2 days following immunization. Mice were examined every day for signs of EAE. The clinical severity was graded into six categories (grade 0, no sign; grade 1, tail paralysis; grade 2, mild hind limb paresis; grade 3, moderate to severe hind limb paresis and/or mild forelimb weakness; grade 4, complete hind limb paralysis and/or moderate to severe forelimb weakness; grade 5, quadriplegia or moribund; grade 6, death).

Analysis of proliferative response and cytokine production

Spleens were obtained from lit/lit, lit/+ , and C57BL/6J mice 32 days after immunization with MOG peptide. Single cell suspension was prepared, and cultured in round-bottom 96-well microtiter plates (Asahi Techno Glass, Tokyo, Japan) at a density of 5 × 10⁵ viable cells/well in a total volume of 200 μl of RPMI 1640 medium (Life Technologies, Gaithersburg, MD), supplemented with 10% FCS (HyClone Laboratories, Logan, UT), 2 mM L-glutamine (Life Technologies), 50 μM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), 100 U/ml gentamicin (Life Technologies). Cells were cultured at 37°C in 100% humidity and 5% CO₂ in the presence or absence of varying concentrations of MOG35–55 peptide or 1 μg/ml Con A (Sigma-Aldrich). For suppression experiments, 1 μg/ml of anti-mouse IgG (whole molecule) Abs conjugated to HRP (Sigma-Aldrich) diluted 1/1000 in PBS-1% Tween 20 was applied and incubated for 2 h at room temperature. After washing, a 100-μl aliquot of each mouse serum diluted 50-fold in PBS-1% Tween 20 was applied and incubated for 2 h at room temperature. After another washing, 100 μl of goat anti-mouse IgG (whole molecule) Abs conjugated to HRP (Sigma-Aldrich) diluted 1/10000 in PBS-1% Tween 20 was added and incubated for 2 h at room temperature. The plates were washed three times and color development of p-nitrophenylphosphate (Sigma-Aldrich) was monitored at 405 nm in an ELISA plate reader.

Results

GHRHR-deficient mice were resistant to the induction of EAE

To address the potential involvement of GHRH in the pathogenesis of EAE, we determined whether EAE can be induced in GHRHR-deficient lit/lit mice. EAE was induced with the synthetic MOG peptide, MOG35–55, which is encephalitogenic in C57BL/6J (H-2b) mice (16). The results shown in Fig. 1 demonstrate that most of the control C57BL/6J mice readily developed EAE. In contrast, no lit/lit mice developed any clinical symptoms of EAE during the entire periods of observation (32 days). The disease severity of individual animals is shown in Fig. 1B. Maximal scores of control C57BL/6J mice were significantly (p < 0.005) different from those of lit/lit mice. The heterozygous lit/+ mice, which do not show dwarf phenotype, developed milder disease compared with control C57BL/6J mice. These results indicate that GHRH is required for the development of EAE.

MOG-specific immune responses in GHRHR-deficient mice were not impaired

Resistance to EAE in lit/lit mice can be due to the reduction of immune response to the MOG35–55 peptide used for EAE induction. To address this issue, we examined whether activation of myelin-specific T cells was normal in lit/lit mice. Splenocytes were collected from MOG35–55 peptide-immunized lit/lit, lit/+ , and control C57BL/6J mice 32 days after immunization and tested in vitro for their proliferation in response to MOG35–55 peptide. As shown in Fig. 2A, splenocytes from lit/lit and lit/+ mice proliferated normally in response to MOG peptide. Moreover, Con A-induced proliferation was also intact in splenocytes prepared from lit/lit mice (Fig. 2B). These results suggest that GHRHR deficiency did not affect the activation of MOG-specific T cells.

Because Abs to the MOG Ag may contribute to demyelinating lesions and to the severity of EAE (17), we examined the level of anti-MOG35–55 Ab in the serum of MOG-immunized lit/lit mice. As shown in Fig. 3, the level of anti-MOG35–55 IgG was lower than that of control C57BL/6J mice.
response to immunized MOG 35-55 peptide. In C57BL/6J mice that were immunized with MOG35-55 peptide, no sign of clinical disease was observed. However, in GHRHR-deficient lit/lit mice, significant clinical disease was observed 5 days after immunization. These results suggest that the resistance of GHRHR-deficient mice to MOG-induced EAE was due to a deficient humoral immune response.

**Discussion**

In the present study, we demonstrated that GHRH is crucial for the induction of EAE. None of GHRHR-deficient lit/lit mice developed any sign of clinically evident disease after active immunization with MOG35-55 peptide. Interestingly, heterozygous lit/+ mice developed mild disease symptoms compared with control C57BL/6J mice, suggesting a dose-related effect of GHRH. Moreover, because lit/+ mice do not show dwarf phenotype, it is unlikely that the resistance of lit/lit mice to EAE induction is a secondary phenomenon experienced by dwarf phenotype. Taken together, these results suggest that GHRH plays a critical role in the pathogenesis of EAE.

GHRH stimulates GH secretion from the pituitary gland. GH, directly or via induction of insulin-like growth factor-1, is implicated in lymphocyte development and function. Earlier observations of GH-deficient mice also suggested a pivotal role of GH in the immune system, because Ames (df/df) and Snell-Bagg (dw/dw) dwarf mice show severe immune deficits. Because lit/lit mice also showed reduced GH secretion and dwarf phenotype, it is conceivable that EAE resistance of lit/lit mice is caused by insufficient immune function. To address this question, we analyzed the immune response of MOG35-55-immunized lit/lit mice. However, the in vitro proliferative response to immunized MOG35-55 peptide was not impaired. Con A-induced proliferation was also normal as compared with control C57BL/6J mice. These results suggest that EAE resistance of lit/lit mice was not due to insufficient T cell response. Serum anti-MOG35-55 IgG levels were increased in lit/lit mice. Therefore, although the Ab response by the B cell is also involved in the development of EAE (21, 22), it is unlikely that EAE resistance of lit/lit mice is due to a decreased B cell response to immunized MOG35-55 peptide. Taken together, these observations suggest that the resistance of lit/lit mice to EAE is not due to the reduced immune response caused by insufficient GH secretion. However, the data presented here do not exclude a possible disease-modifying effect of MOG-specific autoantibodies in EAE. Whether the higher level of MOG35-55-specific Abs is involved in EAE resistance of lit/lit mice remains to be clarified.

The neuroendocrine system has potent effects on the immune system. These two systems can communicate in a bidirectional fashion using a common set of signal molecules and their receptors (23). For example, corticotropin-releasing hormone (CRH), a hypothalamic key regulator of stress response in the hypothalamic-pituitary-adrenal axis, suppresses EAE not only mediated by the hypothalamic-pituitary-adrenal axis, but also by direct effects on the immune system (24). In addition, the proinflammatory effects of CRH in vivo and in vitro have also been reported (25, 26). CRH and its receptor are widely distributed outside the brain including immune cells (27–31). The immune cells can also produce over 20 different neuropeptides such as adrenocorticotropic hormone, which is successful in reducing the intensity and duration of relapses of MS (32, 33). In addition, large scale analysis of gene transcripts in MS lesions revealed that leptin, the melanocortin 4 receptor, and the adrenocorticotropic hormone receptor are elevated at the site of inflammation in the brain (34). These data suggest that the bidirectional interaction between immune and neuroendocrine systems may critically influence the susceptibility of autoimmune diseases.

The expression of GHRH and GHRHR on lymphocytes was previously reported (10–13). In addition, in vitro studies demonstrated that GHRH stimulates proliferation of lymphocytes and inhibits NK cell activity (14, 15) and chemotaxis (35) of human lymphocytes. These data suggest direct involvement of GHRH in immune system. However, the precise roles of GHRH in the regulation of the immune system in vivo are unclear. Although our data presented here suggest the requirement of GHRH in EAE development, the mechanisms by which GHRH is involved in autoimmunity remains unknown. Further investigation of the development of chronic inflammatory disorders regarding the influences of the neuroendocrine system including GHRH-GH axis may provide a clue for understanding the etiology of autoimmune diseases.

**References**


