ABSTRACT

The protein encoded by the Gene Associated with Retinoid-Interferon-Induced Mortality-19 (GRIM-19) is located in the mitochondrial inner membrane and is homologous to the NADH dehydrogenase 1-alpha subcomplex subunit 13 of the electron transport chain. Multiple sclerosis (MS) is a demyelinating disease that damages the brain and spinal cord. Although both the cause and mechanism of MS progression remain unclear, it is accepted that an immune disorder is involved. We explored whether GRIM-19 ameliorated MS by increasing the levels of inflammatory cytokines and immune cells; we used a mouse model of experimental autoimmune encephalomyelitis (EAE) to this end. Six-to-eight-week-old male C57BL/6, IFN\(\gamma\)-knockout (KO), and GRIM-19 transgenic mice were used; EAE was induced in all strains. A GRIM-19 overexpression vector (GRIM19 OVN) was electrophoretically injected intravenously. The levels of Th1 and Th17 cells were measured via flow cytometry, immunofluorescence, and immunohistochemical analysis. IL-17A and IFN\(\gamma\) expression levels were assessed via ELISA and quantitative PCR. IL-17A expression decreased and IFN\(\gamma\) expression increased in EAE mice that received injections of the GRIM19 OVN. GRIM-19 transgenic mice expressed more IFN\(\gamma\) than did wild-type mice; this inhibited EAE development. However, the effect of GRIM-19 overexpression on the EAE of IFN\(\gamma\)-KO mice did not differ from that of the empty vector. GRIM-19 expression was therapeutic for EAE mice, elevating the IFN\(\gamma\) level. GRIM-19 regulated the Th17/Treg cell balance.

Keywords: Gene Associated with Retinoid-Interferon-Induced Mortality-19; Multiple sclerosis; Experimental autoimmune encephalomyelitis; IL-17; IFN\(\gamma\)
INTRODUCTION

The Gene Associated with Retinoid-Interferon-Induced Mortality-19 (GRIM-19) encodes a nuclear protein homologous to the NADH dehydrogenase 1-alpha subcomplex subunit 13 (NDUFA13), and tends to induce apoptosis in interferon (IFN)- and all-trans retinoic acid (RA)-induced tumor cells (1). GRIM-19 was showed that the inhibitor of tumor cells by activating IFN family and retinoic acid (2). GRIM-19 gene is located in chromosome 19 in genomic DNA in human, GRIM-19 binds to STAT3 directly in the cytosol and locates mitochondrion membrane (3,4). GRIM-19 is a protein of the mitochondrial inner membrane, being a component of the five electron transport chain complexes that produce cellular energy. GRIM-19 is closely involved in early embryonic development (5), controlling normal tissue development and suppressing tumor formation (6). GRIM-19 controls cell growth and death by targeting multiple proteins/pathways. Cell death is induced by GRIM-19 overexpression; cell growth is induced when GRIM-19 is suppressed (7). Not only is GRIM-19 associated with the development of tumors including osteosarcoma (8), hepatocellular carcinoma (9), lung cancer (10), cervical cancer (11), and prostate cancer (12) but the protein may also be associated with spontaneous abortion (13).

The etiology of multiple sclerosis (MS), an inflammatory, demyelinating, chronic neurodegenerative disorder of the central nervous system, remains elusive (14-17), but is widely thought to reflect abnormal T cell autoimmune destruction of oligodendrocytes and neurons (18). About 2.3 million people are affected globally; approximately 20,000 die annually (19). MS develops between the ages of 20 to 50 years and is more common in females (20). Although some drugs that slow the progression or alleviate the symptoms, no drugs have been developed to cure and the mechanism of disease onset is still unclear (21,22). The causes of MS include vitamin D deficiency, Epstein-Barr virus infection, intestinal bacterial flora, western diets and tobacco (23). To treat MS, use mesenchymal stem cells, anthracenedione antineoplastic agents, IL-1β inhibitors and α4-integrin humanized Abs (15,24,25). However, there is no clear cure method.

Of the several MS animal models, the autoimmune pathogenesis characteristic of MS is replicated in the model of experimental autoimmune encephalomyelitis (EAE) (26) characterized by the development of Abs targeting central nervous system (CNS) Ags such as the MBP-PLP fusion protein (MP4) and the Myelin Oligodendrocyte Glycoprotein (MOG15-55 peptide) (27). EAE is an inflammatory demyelinating disease of the CNS (28) and serves as the prototype model of T cell-mediated autoimmune disease (29). We used the MOG15-55 peptide to trigger EAE in C57BL/6 mice; this is a Th17 cell-dependent model (30). EAE models can be used to explore the mechanisms potentially involved in autoimmune conditions involving the CNS (31), including disease of the spine. Here, we used an EAE mouse model to determine the clinical significance of GRIM-19 expression and associated interferon production. Myelin-reactive T cells that produce IFNγ, IL-17, and GM-CSF are associated with the disabilities of EAE (32-37). Such disabilities are reduced by lowering the levels of pro-inflammatory cytokines including IFNγ and IL-17 (38-40). We induced EAE in GRIM-19 transgenic (GRIM19 TG) mice and IFNγ-knockout (KO) mice. We found a significant association between GRIM-19 and IFNγ status. We used GRIM-19 gene therapy to treat EAE mice; it may be possible to improve EAE employing such therapy.
MATERIALS AND METHODS

Animals
Six-to-eight-week-old male mice (strains C57BL/6 and IFNγ-KO) were purchased from Jackson Laboratory. Mouse GRIM-19 transgenic mice (C57BL/6 background) were purchased from Macrogen (Seoul, Korea). To establish this mouse line, a GRIM-19 fragment was inserted into the pcDNA3.1+ vector (Invitrogen, Waltham, MA, USA) containing the cytomegalovirus promoter by GenScript Corporation (Piscataway, NJ, USA) and microinjected by Macrogen. All animals were maintained under specific pathogen-free (SPF) conditions with free access to standard mouse chow (Ralston Purina, St. Louis, MO, USA) and water. The Animal Care Committee of The Catholic University of Korea approved the experimental protocol. All experimental procedures were evaluated and carried out in accordance with the protocols approved by the Animal Research Ethics Committee at the Catholic University of Korea (ID number: CUMC-2017-0304-02). All procedures followed the ethical guidelines on animal use.

EAE model
EAE was induced by subcutaneous injection of 500 ng MOG35–55 peptide in incomplete Freund’s adjuvant (Chondrex, Redmond, WA, USA) with 500 ng inactivated Mycobacterium tuberculosis (Difco, Franklin Lakes, NJ, USA) supplemented by intravenous injection of 200 ng pertussis toxin (Sigma, St. Louis, MO, USA) on days 0 and 2. The mice were observed and scored on a scale of 0–5 (with gradations at intervals of 0.5, thus allowing intermediate scores): 0, no clinical signs; 1, loss of tail tone; 2, wobbly gait; 3, hindlimb paralysis; 4, hindlimb and forelimb paralysis; and 5, death. Scoring of pathology was conducted by 2 proficient technicians by a blind test. Each group contained 5 mice and all experiments were repeated 3 or more times.

Injection of GRIM-19
To produce a mouse GRIM-19 overexpression vector (GRIM19 OVN), a mouse GRIM-19 fragment (RefSeq: NM_023312.3) was synthesized by TOP Gene Technologies (Quebec, Canada); the codons were optimized for expression in mammalian cells. The construct was subcloned between the BamHI and XhoI sites of pcDNA3.1+. Mice were intravenously injected with 100 µg of this mouse GRIM19 OVN in 1 mL saline. The vector which was applied control groups was used empty mock vector. All vectors were injected per weekly, a total five injections. The vectors were delivered following hydrodynamic gene delivery (41). Then, mice were sacrificed and analyzed. The sequential experimental process were represented (Fig. 1A).

Immunohistochemistry
Spinal cord tissues were paraffin-embedded and 4-µm-thick sections stained with H&E. The spleens were fixed in 4% (v/v) paraformaldehyde, embedded in paraffin, and 4-µm-thick sections were deparaffinized in xylene and dehydrated in ascending baths of 70%–100% (v/v) ethanol. At least four sections from each tissue were analyzed. Immunohistochemistry employed the Vecta ABC kit (Vector Laboratories, Burlingame, CA, USA). Tissue sections were incubated with primary anti-IL-17A Ab overnight at 4°C, followed by a biotinylated secondary Ab and a streptavidin-peroxidase complex for 1 h. The final color was developed using 3,3-diaminobenzidine (Dako, Carpinteria, CA, USA) and the sections counterstained with Mayer’s hematoxylin. Images were captured by a DP71 digital camera (Olympus, Center Valley, PA, USA) fitted to an Olympus BX41 microscope.
Immunostaining for confocal microscopy
Spleen cryosections (5-µm-thick) were stained with phycoerythrin (PE)-conjugated rat anti-mouse CD4 (45-0042-82; eBioscience, Waltham, MA, USA), PE-conjugated mouse anti-mouse GRIM-19 (sc-365978; Santa Cruz Biotechnology, Santa Cruz, TX, USA), FITC-conjugated rat anti-mouse IL-17A (11-7177-81; eBioscience), FITC-conjugated rat anti-mouse IFNγ (505810; BioLegend, San Diego, CA, USA), FITC-conjugated rat anti-mouse CD25 (102006; BioLegend), and rat allophycocyanin (APC)-conjugated mouse anti-Foxp3 (77-5775-40; eBioscience) Abs overnight at 4°C and the stained sections observed under a Zeiss confocal microscope (LSM 510 Meta; Carl Zeiss, Jena, Germany). Numbering of stained-cells was conducted by 2 proficient technicians by a blind test. Each group contained 5 mice and all experiments were repeated three times.

Western blotting
Lysates were centrifuged, proteins were loaded onto 10% (w/v) polyacrylamide gels, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Invitrogen). Membranes were blocked with 5% (w/v) skim milk in Tris-buffered saline with 0.1% (v/v) Tween-20 (TBST) for 1 h at room temperature. Abs against GRIM-19 and β-actin were added, followed by incubation at 4°C overnight. The membranes were reacted with goat anti-mouse HRP-conjugated Abs. Immunoreactivity was determined using an enhanced chemiluminescence system (Amersham Biosciences, Piscataway, NJ, USA).

Figure 1. The IL-17A level decreased and that of IFNγ increased in mice with EAE that overexpressed GRIM-19 (n=5). (A) Schematic representation of the experiments. (B) Mock and GRIM19 OVN were assessed by western blotting. (C) The clinical scores of mock-injected and GRIM-19-injected mice. (D) After 5 wk, the mice were sacrificed and the splenocytes of the mice were isolated and analyzed. Th17 and Treg cell numbers in splenocyte populations were measured via ex vivo cell flow cytometry. (E) The IL-17A, IL-10, and IFNγ levels (as revealed by ELISA) in the culture media of mouse splenocytes cultured under conditions triggering Th17 differentiation (n=5).

* p<0.05, ** p<0.01.
Quantitative PCR (qPCR) analysis
Total RNA was isolated with the TRIzol reagent (Molecular Research Center, Cincinnati, OH, USA). The concentrations of RNA were measured using a NanoDrop ND-1000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). The 2-µg of RNA was reverse-transcribed into cDNA using a Transcriptor First-Strand cDNA Synthesis Kit (Roche Applied Science, Penzberg, Germany). The levels of mRNA were analyzed by qPCR employing a FastStart SYBR Green Master Mix (Roche Applied Science) and a StepOnePlus kit (Applied Biosystems, Foster City, CA, USA) following the manufacturers’ instructions. The relative mRNA levels were normalized to those of β-actin. The primer sequences are listed in Table 1.

Measurement of 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) by immunofluorescence
Splenocytes were isolated on 48-well plates and pretreated with 100 µm H2O2 for 2 h. After washing with PBS, the cells were stained with JC-1 (Invitrogen) solution for 30 min at 37°C and washed 3 times with PBS. The fluorescence intensity was detected by a CytoFluor multiwell plate reader at 514 nm for excitation and 529 nm for emission for green (monomer form) fluorescence, and 585 nm for excitation and 590 nm for emission for red (aggregate form) fluorescence.

Measurement of intracellular ROS and mitochondrial superoxide production
Mitochondrial superoxide levels were measured by mitoSOX RED staining according to the manufacturer’s instructions. The splenocytes were treated with DOX and BAY60-2770, and then incubated with 2 µM of mitoSOX RED for 30 min at 37°C. After washing with PBS, red fluorescence was quantified with a fluorescence reader at excitation/emission wavelengths of 510/580 nm.

Flow cytometry
Cells that were for analysis of Th1 and Th17 population were stimulated with PMA and ionomycin with the GolgiStop for 4 h (BD Biosciences, San Jose, CA, USA). To quantify Th1, Th17, and Foxp3-positive Treg cells, splenocytes were immunostained using a PerCp-conjugated anti-CD4 Ab (eBioscience) and fixed and permeabilized using a Cytofix/Cytoperm Plus kit (BD Biosciences). Following the manufacturer’s instructions, splenocytes were stained with FITC-conjugated anti-IL-17A and APC-conjugated anti-IFNγ Abs (eBioscience). To identify Treg cells, splenocytes were surface-labeled with PerCp-conjugated anti-CD4 and APC-conjugated anti-CD25 Abs, followed by fixation, permeabilization, and intracellular staining with a PE-conjugated anti-Foxp3 Ab. All cells were detected using a FACS Calibur device (BD Pharmingen, Franklin Lakes, NJ, USA).

T-cell isolation, Th17 differentiation, and ELISAs
To determine the levels of IFNγ, IL-17A, and IL-10 expressed under conditions of Th17 differentiation, murine splenocytes were cultured in RPMI 1640 medium supplemented with 5% (v/v) FBS. CD4-positive T cells were sorted employing CD4-coated magnetic beads and a magnetically activated cell sorting (MACS) separation column (Miltenyi Biotec, Bergisch Gladbach, Germany). CD4-positive T cells were stimulated by addition of anti-CD3 (0.5 µg/mL).
and soluble anti-CD28 Abs (1 μg/mL; both from BD Biosciences), anti-IFNγ (2 μg/mL) and anti-IL-4 (2 μg/mL) Abs (Invitrogen), recombinant TGF-β (2 ng/mL), and recombinant IL-6 (20 ng/mL) (R&D Systems, Minneapolis, MN, USA) for 3 days. Culture supernatants were subjected to sandwich ELISAs (R&D Systems). Alkaline phosphatase (Sigma) was used for color development. Absorbance was determined at a wavelength of 405 nm using an ELISA microplate reader (Molecular Devices, San Jose, CA, USA).

**Statistical analysis**

All between-group comparisons were made using the nonparametric Mann-Whitney U test; all among-group (3 or more) comparisons were conducted via 1-way analysis of variance with Bonferroni’s post hoc test. GraphPad Prism software (ver. 5.01) was employed for all analyses. A p-value <0.05 was considered statistically significant. Data are expressed as means±SDs.

**RESULTS**

**The severity of EAE pathology was reduced by GRIM-19 overexpression via reduction of IL-17A levels**

First, the pcDNA3.1+ hosting GRIM-19 (GRIM19 OVN) vector and mock vector were injected to EAE mice for 5 wk at 1-wk intervals (Fig. 1A). GRIM19 OVN vector and mock vector were transfected in HEK293 cells. Then, the lysates of cells were determined by western blotting for verifying of overexpression of GRIM-19 (Fig. 1B). To explore whether GRIM-19 overexpression reduced EAE pathology, we injected GRIM19 OVN into EAE mice. The clinical score was significantly lower in GRIM19 OVN- than mock vector-injected mice (Fig. 1C). Splenocytes were subjected to flow cytometry (Fig. 1D). The proportion of Th1 cells was significantly increased and that of Th17 cells markedly decreased in GRIM19 OVN-injected mice. Under conditions of Th17 differentiation, the IL-17A level fell in GRIM19 OVN injected-mice and that of IL-10 increased. Although the IFNγ level increased somewhat in the former mice, the between-group difference was not significant (Fig. 1E). Thus, GRIM-19 overexpression ameliorated EAE pathology.

**GRIM-19 overexpression was therapeutically efficacious**

To investigate the lymphocyte transition, spleen cryosections were stained using anti-GRIM-19, -IL-17A, -CD25, and -Foxp3 Abs for immunofluorescence analyses. In GRIM19 OVN mice, GRIM-19 expression was significantly elevated in splenic lymphocytes and the Th17 population was significantly decreased. However, the Foxp3-positive Treg cell numbers did not differ significantly between mice injected with the empty vector and the GRIM19 OVN construct (Fig. 2A). Lymphocyte infiltration into the spinal cord of mock-injected mice was higher in the former than the latter mice (Fig. 2B). The paraffin sections revealed spinal cord damage. The spinal cord surface of empty vector-injected EAE mice was damaged by lymphocyte infiltration; the spinal cord of mice injected with the GRIM19 OVN construct was not. Immunohistochemical images revealed lower IL-17A expression in the spinal cord of GRIM19 OVN than empty vector-injected mice (Fig. 2C). The H&E staining data showed the hippocampal neurons in the mock injected mice and GRIM19 OVN injected mice (Fig. 2D). The neurons of in the GRIM19 OVN group exhibited clear cell layer compared to mock group. Thus, GRIM-19 overexpression reduced spinal cord and brain damage in EAE mice.
EAE pathology was inhibited in GRIM TG mice via elevation of the IFNγ level and Treg cell numbers

We used GRIM19 TG mice to determine whether GRIM-19 overexpression inhibited the development of EAE symptoms. First, we established GRIM19 TG mice for experiments. GRIM-19 protein level was higher than WT mice in GRIM19 TG mice (Fig. 3A). The EAE clinical score of GRIM19 TG mice was significantly lower than that of the C57BL/6 EAE mice (Fig. 3B). The lymphocytes of the spleen and draining lymph nodes (dLNs) of each mouse were analyzed via flow cytometry. The populations of Treg cells were markedly higher in the spleen and dLNs of GRIM19 TG mice than control mice (Fig. 3C). In splenocytes, the Th1 cell population was significantly increased and Th17 level was reduced in GRIM19 TG mice (Fig. 3D). The serum levels of total IgG, IgG1, and IgG2a were significantly lower in GRIM19 TG mice than control C57BL/6 EAE mice (Fig. 3E). The population of IL-17 positive cells was
decreased in GRIM19 TG mice compared to WT (Fig. 3F). The H&E staining data showed the hippocampal neurons, the neurons of in the GRIM19 TG mice exhibited clear cell layer compared to WT mice (Fig. 3G). Thus, GRIM-19 inhibited EAE pathology by increasing the IFNγ level.

The mitochondrial function was improved in GRIM19 TG mice

To determine which difference has inhibition of EAE development in GRIM19 TG mice, we considered mitochondria change in GRIM19 TG mice to be different and investigated mitochondrial functions. Although there were no significant differences, the mitochondrial membrane potential was improved in GRIM19 TG mice through JC-1 staining (Fig. 4A). The population of mitochondria of splenocytes was increased in GRIM-19 TG mice (Fig. 4B). Besides, the mitochondrial superoxide was decreased in GRIM-19 TG mice significantly (Fig. 4C). The immunofluorescence images of transgenic mice showed a higher GRIM-19 expression of CD4-positive cells compared to WT mice (Fig. 4D). Therefore, this data suggested that the improvement of mitochondrial functions of GRIM19 TG mice may affect CD4-positive T cells.

EAE pathology was not inhibited in IFNγ-deficient mice injected with the GRIM-19 vector

To explore whether GRIM-19 overexpression reduced EAE pathology by elevating the IFNγ level, we established an IFNγ-KO mouse line. In such mice, neither the GRIM19 OVN construct nor the empty vector affected the clinical EAE score (Fig. 5A). The Th17 cell population was increased in the spleen of GRIM19 OVN injected IFNγ-KO mice, but the Treg cell population was not (Fig. 5B). Flow cytometry data of splenocytes showed that the population of Th1 and Th17 cells was slightly increased in GRIM19 OVN injected IFNγ-KO mice. However, there were no significant differences (Fig. 5C). The levels of mRNAs encoding IFNγ and IL-17A were measured via qPCR. The level of mRNA encoding IFNγ was markedly increased and that encoding IL-17A decreased in GRIM19 OVN injected WT mice. However, the mRNA expression of IFNγ and IL-17A of IFNγ-KO mice has no significant differences between mock and GRIM10 OVN injection groups (Fig. 5D). Thus, GRIM-19 overexpression did not affect EAE symptoms under IFNγ-deficient conditions.

DISCUSSION

We showed that GRIM-19 inhibited EAE progression. GRIM-19 overexpression reduced the Th17 population in EAE mice. Notably, GRIM-19 inhibited EAE development only in the presence of IFNγ. In previous studies, GRIM-19 increases interferon family as cell death regulatory protein through inhibition of STAT3 (1,4). GRIM-19 elevates the IFNβ and combination of retinoic acid, it regulates cell death related genes (42). GRIM-19 overexpression increased the IFNγ level to alleviate EAE pathology in EAE mice. GRIM-19 is a subunit of an NADH dehydrogenase that plays a critical role in the mitochondrial inner membrane (43). Although GRIM-19 was first used to inhibit cancer cell proliferation, recent studies found that GRIM-19 played roles in chronic inflammatory diseases including Crohn’s disease and inflammatory bowel disease; GRIM-19 expression was reduced in such patients (44). GRIM-19 acts as an anti-bacterial regulator in the context of CARD15-mediated innate mucosal responses; GRIM-19 modulates the intestinal epithelial cell responses to microbial infection (45). However, it is unclear how GRIM-19 regulates inflammation. Thus, we explored the relevance of GRIM-19 in another inflammatory disease, EAE.
We previously showed that GRIM-19 reduced progression of inflammatory bowel disease, graft-versus-host disease, and autoimmune arthritis, by regulating Th17 and Treg cell numbers (46-48). To explore whether GRIM-19 overexpression regulated the clinical pathology of EAE
mice, we injected a GRIM-19 overexpression construct (32) and the empty vector into EAE mice via intravenous electrophoresis. Notably, the IFN\(\gamma\) level increased and that of IL-17A decreased in such mice. These data were confirmed *in vitro* using splenocytes that were Th17-differentiated. Lymphocyte infiltration into the spinal cord was reduced in GRIM19 OVN-injected mice; GRIM-19 protected the spinal cord by inhibiting lymphocyte infiltration.

We used GRIM19 TG mice to explore whether GRIM-19 overexpression delayed EAE development. The EAE clinical score of such mice was much lower than that of control mice. In the former animals, the numbers of splenic IFN\(\gamma\)-expressing CD4-positive T cells increased. Although the IL-17A levels did not differ significantly between the groups, the level was somewhat lower in GRIM19 TG mice (Fig. 3D). Treg cell numbers increased in the spleen and dLNs of GRIM19 TG mice, and the serum IgG level decreased. Together, the data indicate that GRIM-19 overexpression regulated the immune response of EAE mice by modulating Th17 and Treg cell numbers. In both GRIM19 OVN-injected and GRIM19 TG mice, the IFN\(\gamma\) level was elevated.

We previously showed that an increased IFN\(\gamma\) level retarded the progression of rheumatoid arthritis (an autoimmune disease) by reducing Th17 cell numbers (49). Rheumatoid arthritis progression was more rapid in IFN\(\gamma\)-KO mice (50). IFN\(\gamma\) affects the innate and adaptive immune cells of EAE mice differently (51). The mechanism of IFN\(\gamma\) remains unclear. Although IFN\(\gamma\) has two-faced function in autoimmune diseases, induction of IFN\(\gamma\) has protective effect in EAE mouse (52) and decreases the level of IL-17A (51). IFN\(\gamma\) deficient mice which were IFN\(\gamma\) KO and anti-IFN\(\gamma\) Ab injected mice had severe EAE pathology and

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*Figure 4.* GRIM-19 overexpression induces the mitochondrial functions. (A) Mitochondrial membrane potential was detected using JC-1 dye for flow cytometry. (B) The confocal microscopy images showed the number of mitochondria in mice splenocytes. (C) The mitochondrial superoxide of splenocytes was measured by mitoSOX Red staining production assay. (D) The immunofluorescence images exhibited the CD4 and GRIM-19 double-positive cells.

\^{p<0.05.}
an increase of immune cells in IFN gamma KO mice and anti-IFN-gamma injected mice (53). Especially, the treatment of IFNγ in EAE mice decreased clinical symptoms under the conditions that type I (IFNα and IFNβ) IFN exists (54).

Some studies found that GRIM-19 expression affected interferon levels (5,55). We hypothesized that GRIM-19 expression might correlate to that of IFNγ; we explored this possibility using IFNγ-KO mice. As expected, GRIM-19 overexpression exerted no therapeutic effect in IFNγ-KO EAE mice. Th1 cell numbers were reduced in the spleen tissue cells and splenocytes of IFNγ-KO mice, but the Th17 cell numbers increased. At the mRNA level, IFNγ
expression decreased and that of IL-17A increased in IFNγ-KO mice. We found that GRIM-19 inhibited the development of EAE in mice by regulating the levels of IFNγ and IL-17A and protecting the spinal cord against lymphocyte infiltration.

The GRIM-19 study focused primarily on inhibiting cancer cell growth (56,57). In particular, it is widely known to inhibit tumors through the increase of the IFN family (13,58). Our study suggested that GRIM-19 overexpression decreased the pathology of EAE symptoms through elevating of IFNγ levels. Overexpression of GRIM-19 reduced the level of IL-17A. Taken together, these results showed that the GRIM-19 expression has potentially therapeutic in autoimmune disease patients. However, we have not shown how GRIM-19 regulates IL-17A and IFNγ in detail mechanism. Further work is required.

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