progesterone in autoimmune diseases (14, 15). The gender difference was also observed in the development of EAE in SJL/J mice (16). Treatment with estrogen, at high doses such as those achieved during pregnancy, ameliorates EAE and other CD4+ Th1 cell-mediated autoimmune diseases (17). Although the inhibition of EAE by estrogen associates with a decrease in the expression of proinflammatory cytokines (18), the exact mechanisms involved in the regulation of EAE/MS by estrogen and other sex hormones are not well defined. The sex steroid hormones, produced primarily in the ovary and testis, circulate in the blood, diffuse into immune cells, and activate specific nuclear receptor transcription factors. The estrogen receptors belong to a large family of nuclear receptor transcription factors (19). Upon ligand binding, these receptors form homodimers or heterodimers (ERα, ERβ), translocate into the nucleus, and interact with specific DNA elements and transcription factors (20). The estrogen receptor complex can also interact with and inhibit kinases and transcription factors that mediate immune and inflammatory responses (21). Through this mechanism, estrogen may regulate Th1/Th2 balance by influencing IL-12/IFNγ axis in EAE/MS.

Phytoestrogens are chemically diverse group of flavonoid compounds, produced as plant metabolites that can have estrogenic effects in animals (22–26). The dietary flavonoids have potent antioxidant effects and exert antitumor, -allergic, -inflammatory, and -viral activities (27–29). Quercetin (3,3′,4′,5,7-pentahydroxy flavone) is a flavonoid phytoestrogen abundantly present in soybeans, vegetables, and fruits. Quercetin is a potent inhibitor of tumor growth and inflammation and has traditionally been used to treat many inflammatory disorders (30–33). Although estrogen treatment has been shown to inhibit EAE, no study has hitherto examined the use of phytoestrogens in the treatment of EAE/MS. In this study, we have examined the effect and mechanism of action of quercetin in EAE. Our results show that quercetin inhibits EAE by blocking IL-12 production, IL-12 signaling, and Th1 differentiation, suggesting its use in the treatment of MS and other Th1 cell-mediated autoimmune diseases.

MATERIALS AND METHODS

Animals and Cells

SJL/J mice were purchased from the Jackson Laboratory (Bar Harbor, Maine) and maintained in the animal care facility at Vanderbilt University Medical Center. Activated T cells were prepared by stimulation of spleen cells from SJL/J mice (2 × 10^6/mL) with 5 µg/mL of Concanavalin A (ConA, Pharmacia Biotech, Uppsala, Sweden) in RPMI-1640 medium supplemented with 10% FBS (Gibco BRL, Rockville, MD) at 37°C and 5% CO2. After 3 days of culture, cells were harvested and cultured in medium containing 0.5% FBS for an additional 24 h to synchronize to G1 phase of the cell cycle. The T cell blasts were isolated by centrifugation over Histopaque (Sigma, MO) at 1200g for 15 min and used for experiments (34, 35). This population of cells normally contains >98% T cell blasts as measured by flow cytometry. The peritoneal macrophage was isolated from thioglycollate-stimulated SJL/J mice as described elsewhere (36, 37). The EOC-20 mouse microglial cell line (38) was a kind gift of W. Walker (St. Jude Children’s Research Hospital, Memphis, TN).

Reagents

Quercetin (3,3′,4′,5,7-pentahydroxy flavone) was purchased from Calbiochem (La Jolla, CA). Recombinant murine IL-12 and IFNγ were purchased from R&D Systems, Inc. (Minneapolis, MN). The anti-IFNγ mAb, R46A2 was purified from ascetic fluid collected from nude mice following transplantation of R4 6A2 hybridoma cells (American Type Culture Collection # HB 170, Rockville, MD). The anti-IFNγ mAb, MM700 was obtained from Endogen (Woburn, MA) and conjugated with biotin according to standard protocol. Anti-IL-12 mAb C17.8 (anti- p40) was prepared from hybridoma cells kindly provided by G. Trinchieri (Wistar Institute, Philadelphia, PA). Mouse spinal cord homogenate (MSCH) and Guinea pig MBP were prepared according to standard protocols (39). Anti-JAK2 Ab and anti-phosphotyrosine mAb 4G10 were purchased from Upstate Biotechnology, Inc. (Lake placid, NY). Anti-TYK2, anti-STAT3 and anti-STAT4 Abs were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

Induction and Treatment of EAE

To induce EAE, 4–6 weeks old female SJL/J mice were immunized (s.c.) with 800 µg of MSCH in 150-µL emulsion of incomplete Freund’s adjuvant containing 50-µg/mL H37Ra in the lower dorsum on days 0 and 7. Mice in the test groups were treated (i.p.) with 50- or 100-µg quercetin in 25-µL DMSO on every other day from 0 to 25 days following induction of active or passive EAE. Mice in the control group received 25-µL DMSO. The clinical symptoms of paralysis in EAE was graded on a daily basis in a blinded manner as follows: 0, normal; 0.5, stiff tail; 1, limp tail; 1.5, limp tail with inability to right; 2, paralysis of one limb; 2.5, paralysis of one limb and weakness of one other limb; 3, complete paralysis of both hind limbs; 4, moribund; 5, death (7, 8, 39).

Mean clinical