score (MCS) was calculated by adding every daily clinical score for all mice in a group and then divided by total number of mice. Mean maximum clinical score (MMCS) was the MCS at the peak of disease.

**Histological Analysis**

To assess the degree of CNS inflammation and demyelination, mice treated with quercetin following induction of active EAE were euthanized on day 15 (at the peak of disease) by CO2 asphyxiation and perfused by intracardiac injection of 4% paraformaldehyde and 1% glutaraldehyde in PBS. Transverse sections (five each) taken from different levels of cervical, upper thoracic, lower thoracic, and lumbar regions of the spinal cord were stained with Luxol Fast Blue or hematoxylin and eosin. The pathology of inflammation and demyelination in the CNS was examined under microscope in a blinded manner. The spinal cord sections were viewed as anterior, posterior, and two lateral columns (four quadrants). Each quadrant displaying the infiltration of mononuclear cells or loss of myelin staining was assigned a score of one for inflammation or one demyelination respectively. Thus, each animal had a potential maximum score of 16 and this study represents the analysis of spinal cord from 10 representative mice per group. The pathologic scores for each group are expressed as percent positive over the total number of quadrants examined (7, 8, 39–43).

**T Cell Proliferation Assay**

The effect of quercetin on neural antigen- or IL-12-induced T cell proliferation was measured by ³H thymidine incorporation assay. MBP-immune spleen cells were cultured in 96 well tissue culture plates in RPMI medium (2 × 10⁵/200 µl/well) in the presence of 0 or 25 µg/mL MBP with different concentrations (0–10 µg/mL) of quercetin. ³H thymidine (0.5 µCi/well) was added at 72 h and the uptake of radiolabel measured after 96 h by Wallac beta plate scintillation counter (7, 8, 39–42). MBP-immune spleen cells were also cultured with 0 or 25 µg/mL MBP and 2 ng/mL rIL-12 with different concentrations (0–10 µg/mL) of quercetin. ³H thymidine (0.5 µCi/well) was added at 72 h and the uptake of radiolabel measured as above after 96 h. ConA-activated T cells were cultured in RPMI-1640 medium in 96 well tissue culture plates (1 × 10⁵/well) with 2 ng/mL rIL-12 in the absence or presence of different concentrations of quercetin (0–10 µg/mL) for 48 h. ³H thymidine was added for the last 12 h and the radiolabel measured as above (7, 8, 34, 35, 39–43).

**Immunoprecipitation and Western Blot Analysis**

The immunoprecipitation and Western blot analysis of JAK and STAT proteins were performed as described earlier (7, 8, 34, 35, 42). Briefly, ConA-activated T cells (2.5 × 10⁵) were pretreated with different concentrations of quercetin (0–25 µg/mL) for 15 min and then stimulated with 5-ng/mL IL-12 at 37°C for 15 min. Cell lysates were prepared and the JAK2, TYK2, STAT3, and STAT4 proteins immunoprecipitated using specific antibodies and protein A Sepharose. The phosphoproteins in the immune complexes were analyzed by 7.5% SDS-PAGE and Western blot using antiphosphotyrosine mAb 4G10 and visualized by enhanced chemiluminescence (ECL) detection system. The blots were stripped and reprobed with specific Ab to ensure equal protein loading.

**Culture for IL-12 and IFNγ Assay**

MBP-immune spleen cells were cultured in 24 well plates in RPMI-1640 medium (5 × 10⁴/mL) with 25-µg/mL MBP in the presence of different concentrations of quercetin (0–10 µg/mL) and the culture supernatants were collected after 24 h. Peritoneal macrophage and EOC-20 microglial cells were cultured in DMEM containing 10% FBS with 50-ng/mL rIFNγ and 1-µg/mL LPS in the absence or presence of quercetin and the culture supernatants were collected after 48 h (36–38). Naïve spleen T cells from SJL/J mice were enriched (>95% purity) by passing through nylon wool column and plastic adherence. Anti-CD3 mAb (2C11, 5 µg/mL) was immobilized onto 6 well tissue culture plates by incubation for 1 h at 37°C. After washing the plates with PBS, T cells were added into the wells (1 × 10⁶ cells/well) and cultured in the presence or absence of 2-ng/mL rIL-12 and different concentrations of quercetin. After 5 days of culture, equal number of viable cells (5 × 10⁶ cells/mL) were restimulated with soluble anti-CD3 mAb (2C11, 5 µg/mL) and the supernatants were collected after 36 h (7, 8, 34, 35, 41–43).

**ELISA for IL-12 and IFNγ**

The levels of IL-12 and IFNγ in the culture supernatants were measured by ELISA as described earlier (7, 8, 34, 35, 40–42). Briefly, ELISA plates were coated with 2 µg/mL of anti-IL-12 mAb, C17.15 or anti-IFNγ mAb, R4-6A2 capture Ab in 100 µL/well of carbonate buffer, pH 9.3. After overnight incubation at 4°C, excess Ab was washed off and the residual binding sites were blocked by the addition of 3% BSA in PBS for 1 h. The test samples (culture supernatants and standards (rIL-12 or rIFNγ) were added and incubated overnight at 4°C. Plates were washed with...