Title: Characterizing the immunomodulatory effects of the antioxidant TEMPOL in a model of multiple sclerosis

Background: Reactive oxygen and nitrogen species are implicated in inflammatory-mediated damage to the central nervous system in multiple sclerosis (MS) and an animal model of the disease, experimental autoimmune encephalomyelitis (EAE). We have shown that oral administration of the antioxidant TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a stable nitroxide radical, lowers incidence and reduces severity of disease in EAE. We hypothesize that TEMPOL limits inflammatory demyelinating disease by regulating the development of pathogenic immune responses that influence immune cell activation, including T cell and antigen presenting cell phenotypes and function.

Methods: Immune responses were compared between control and TEMPOL-fed EAE or healthy mice by examining differences in proliferation, population distribution, surface marker expression, and cytokine production in immune cells isolated from lymphoid organs. The effect of added TEMPOL on immune cell proliferation and phenotype was also studied *in vitro* using mixed lymphocyte reactions (MLR) with human or mouse cells, and in isolated murine lymphoid cell cultures stimulated with anti-CD3.

Results: TEMPOL-fed animals exhibit comparable levels of myelin-reactive T cells versus controls, but show reduced production of the pro-inflammatory cytokines interferon gamma, tumor necrosis factor alpha, and transforming growth factor-beta 1. Flow cytometry showed enrichment of CD8+ over CD4+ T cells in lymphoid tissues of TEMPOL-fed EAE mice, as well as decreased MHC II and increased CD80 and CD86 expression in myeloid cells and myeloid dendritic cell (DC) populations. Enrichment of Foxp3+ regulatory T cells was also observed in lymph nodes with TEMPOL. *In vitro*, TEMPOL was found to enhance proliferation of lymphoid cells in mouse MLR or when stimulated with anti-CD3 in a dose-dependent manner. Human MLR experiments also showed enhanced cell proliferation and enrichment of CD8 T cells in the presence of TEMPOL. TEMPOL decreased expression of MHC II, CD80, and CD86 in splenic myeloid cells and myeloid DCs.

Conclusions: These studies suggest that TEMPOL is not globally immunosuppressive, but instead alters the phenotype of antigen-specific or autoreactive immune cells generated *in vivo*, reducing the pro-inflammatory nature of immune responses in EAE. These immunomodulatory properties contribute to TEMPOL’s potential as an efficacious therapeutic in MS.