

Role of innate immunity in the neuroprotective effect of estrogens

E. Vegeto, G. Calderazzi, G. Pepe, A.M. Villa, A. Maggi

Center of Excellence on Neurodegenerative Diseases, Dept. of Pharmacological and Biomolecular Sciences, University of Milan

Activation of the brain inflammatory response plays a key role in the pathogenesis and progression of neurodegenerative diseases, such as Parkinson's disease (Glass et al, 2010), although the specific contribution of pro and anti-inflammatory phenotypes of microglia is still unclear. Several lines of evidence show a different female/male ratio in the incidence of neurodegenerative diseases, including PD, which has been at least partially ascribed to the neuroprotective activity of estrogen hormones (Bouman et al, 2005). Although it is known that there is a gender-related dimorphism in innate immunity, which drives the inflammatory response, the interplay between the neuroprotective effects of estrogens and hormone action in inflammatory cells is still poorly understood. Our previous data showed that 17 β -estradiol (E2) is able to reduce the pro-inflammatory response of the brain induced by LPS, a potent inflammatory stimulus, or by amyloid deposition in the APP23 mice (Vegeto et al, 2006). Thus, the aim of the study is to better characterize the response of macrophage cells to estrogens, by analyzing gene expression and cell polarization through a genome wide approach, and to evaluate the relevance of dampening neuroinflammation for the efficacy of neuroprotective strategies by using an experimental model of PD. Female mice were treated subcutaneously with vehicle or 5 μ g/kg E2 for 4 hr. Peritoneal macrophages were isolated by magnetic beads preloaded with an antibody against CD11b, RNA extracted and assayed for gene expression by realtime PCR. E2 treatment resulted in an increase in selected mRNAs, such as Tgm-2 and ApoE, known to be under estrogen control in other tissues, thus showing that peripheral macrophages in the intact animal are responsive to this hormone. The hormonal responsiveness of microglia cells is underway as we recently optimized the isolation of microglia cells from the adult mouse brain. Polarization of microglia cells was first analyzed by setting up a protocol of intracerebroventricular (icv) injections of IL-4, a well-known inducer of the M2 phenotype, followed by gene expression and immunological analyses of known M2 markers. Results will be presented.

In summary, we observed that estrogens are able to modify the gene expression programme of macrophage cells *in vivo*, corroborating the hypothesis that these hormones are key regulators of the inflammatory response. These preliminary results sustain further analyses of hormone action in neuroinflammatory cells and in experimental models of neurodegenerative diseases associated with inflammation and macrophage polarization.

Glass et al. (2010) *Cell* 140: 918-34.

Bouman et al. (2005) *Hum Reprod Update* 11: 411-23.

Vegeto et al. (2006) *Endocrinology* 147:2263-72.