Anti-Pyroptotic Effects of Dimethyl Fumarate: an *In Vitro* Study

G. Miglio[1], M. Rogazzo[1] and R. Fantozzi[1]

[1]Dip. di Scienza e Tecnologia del Farmaco, Università di Torino, Italy

Dimethyl fumarate (DMF) is the active component of a defined mixture of fumaric acid esters (Fumaderm®), which has been registered since 1994 for the oral treatment of *psoriasis vulgaris* only in Germany. On March 2013, a new oral formulation of DMF (Tecfidera™) has been approved by the U.S. Food and Drug Administration for the treatment of adult patients with relapsing-remitting multiple sclerosis. A positive opinion, recommending the granting of a marketing authorization, has been also adopted by the Committee for Medicinal Products for Human Use of the European Medicines Agency. Despite numerous studies, the mechanism of action of DMF remains not fully understood. It has been shown that DMF inhibits IL-1β release from cells of the monocyte/macrophage lineage and this effect was related to the inhibition of NF-κB activity. However, as the release of IL-1β is a multi-step process, we have hypothesized that additional actions could underlie the anti-IL-1β effects exerted by this drug. The study was aimed to establish whether DMF and its metabolite monomethyl fumarate (MMF) inhibit pyroptosis, which is a form of programmed cell death highly inflammatory and related to the production of IL-1β. Phorbol myristate acetate-differentiated THP-1 cells were stimulated with bacterial lipopolysaccharide (LPS), then pulsed with ATP in serum-free medium. Cells were exposed to increasing concentrations (0.1-100 μM) of DMF or MMF, 15-60 min before the ATP pulse. Cell death was evaluated by measuring the LDH activity in the collected supernatants. The LPS/ATP-triggered cell death was significantly decreased by both DMF and MMF, in a time- and concentration-dependent manner. These inhibitory effects were comparable to those of parthenolide, which is a natural sesquiterpene lactone endowed with anti-pyroptotic activity. Together with previous findings, our results indicate that DMF is an immunomodulating drug with multiple targets. It is conceivable that by perturbing the activity of several nodes, it causes a fail-on failure of the signaling network underling immune responses.