DIFFERENT PHENOTYPES OF MICROGLIA ACTIVATION

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Chronic inflammation is a hallmark of several neurodegenerative diseases, although the etiology of this process is still poorly characterized. Glial cells, particularly microglia, have been shown to play an important role in sustaining inflammation within the brain. Microglia are normally present in the adult brain in a resting state, and their activation is characterized by secretion of a wide spectrum of proinflammatory molecules (cytokines and prostanoids) and by the release of cytotoxic substances, such as reactive oxygen species and nitric oxide (NO). Under physiological conditions, NO is produced by the constitutive forms of NO synthase (NOS), at low levels and in a regulated manner, acting as a transient signaling agent. However, microglia respond to injury and inflammatory stimuli by upregulation of inducible NOS (NOS2), producing NO at high rate and for prolonged times thus contributing to neuronal damage. Therefore, microglia activation has been usually considered a detrimental factor. Interestingly, recent data support a possible role of reactive microglia in neuroprotection, particularly against the damaging effects of sustained inflammation. Taken together these data suggest that there may be several phenotypes of microglia activation, which can explain both detrimental and beneficial effects. Most of the data obtained *in vitro* on microglia activation have been collected using the bacterial endotoxin LPS as proinflammatory stimulus. LPS triggers a robust activation of microglia which may not be representative of the more complex *in vivo* situation. With this in mind, we tested the effects of proinflammatory cytokines (IFNγ, TNFα, and IL1β) on primary cultures of rat cortical microglia, using LPS for comparison. As markers of microglia activation, we evaluated the expression of NOS2 and its activity, indirectly by assessment of nitrite production in the incubation media; and the expression and activity of inducible cyclo-oxigenase (COX2). While low doses of LPS (1 ng/ml) increased both nitrite and prostaglandin (PG)-E2 production after 48 hour incubations, only the cytokine mixture containing 10 IU/ml IFNγ,10 ng/ml TNFα, and 10 ng/ml IL1β showed similar effects. Increased nitrite production in microglia was due to upregulation of NOS2, as showed by mRNA analysis using real time PCR. Despite production of proinflammatory and cytotoxic molecules, microglia exposed to the cytokine showed higher cell viability, which suggest that cytokines may also increase cell proliferation. When used alone, IFNγ slightly increased nitrite production, while IL1β showed modest stimulatory effects on PGE2, further supporting the hypothesis that different states of microglia activation may occur *in vitro* as well as *in vivo*.