**Abstract**

Information contributes to such neurodegenerative disorders as Alzheimer’s and Parkinson’s diseases. Activated microglial cells surround the plaques and Lewy bodies in Alzheimer’s and Parkinson’s diseases, which is a major component of Lewy bodies, in combination with interferon (IFN)-α/γ induced an inflammatory state in humans. TNF-α, a pro-inflammatory cytokine, was induced in response to alpha-synuclein. To investigate the signaling mechanism of alpha-synuclein treatment of THP-1 cells, profiling with antibodies for over 100 key regulatory phospho-sites was undertaken. At least 10 target phospho-sites were detected. TNF-α and IFN-α increases in activating phospho-sites of MAP kinase (Thr83/Y84), JNK MAP kinase (Thr183/Y185), p38 MAP kinase (Thr180/Y182) and PKC (Thr66) were induced. In parallel, the phosphoforms of many proliferation-associated proteins including p53/p21Cip1/p36, Bcl-2/bax, GSK3β, Akt and S6P were reduced by greater than 40%. These findings demonstrate that alpha-synuclein acts as a potent activator of microglial cells and could unravel the toxic role of mutant forms of alpha-synuclein. Intention of microglial activation by alpha-synuclein could be beneficial in the treatment of Parkinson’s disease.

**Introduction**

The physiological functions of alpha-synuclein (α-syn) have not been entirely defined. Much attention has been focused on the possible involvement in neurodegenerative stress-related mutations are a causative factor in Parkinson’s disease (PD). Currently there are three known point mutations ASP39, ASP41 and ASP39 that result in PD. Aggregated α-syn is a major component of Lewy bodies, which are hallmark of PD and Lewy body dementia. The cellular and molecular mechanisms underlying the pathological action of α-syn are not fully understood. While most studies have concentrated on the effects of α-syn on neuronal cells, recent observations have also pointed towards an interaction between α-syn and inflammatory cytokines. Many factors that are secreted from cells under pathological conditions. Accumulation of extracellular α-syn due to leakage from damaged cells or in vivo of secretion might have pathogenetic consequences. We report here that the disease-causing mutations of α-syn, ASP39, ASP41 and ASP39 are more potent than normal α-syn in inducing neurotoxicity and apoptosis of two pro-inflammatory cytokines, interleukins-1β (IL-1β) and tumor necrosis factor (TNF-α). Profiling with antibodies for over 100 key regulatory protein phospho-sites revealed that α-syn stimulates the p38, JNK and ERK1/2 kinases and activated protein MAP kinase pathways in a receptor-mediated fashion. By contrast, cell cycle signalling pathways involving mitogen-dependent kinase 1 were inhibited.

**Results**

We initially assessed the effects of wild-type and disease-causing mutants of human α-syn to induce THP-1 cell toxicity towards SH-SY5Y neuroblastoma cells when combined with IFN-α. THP-1 cells were incubated with various concentrations (shown on the abscissa of Figure 1) of α-syn in the absence (A) or presence (B) of 100 U/ml IFN-α for 24 h. The wild-type as well as ASP39, ASP41 and ASP39 mutated forms of α-syn were used without aggregation. Subsequently, the cell-free supernatants of THP-1 cells were transferred to the cells containing SH-SY5Y cells and the viability of SH-SY5Y cells and the viability of SH-SY5Y cells was assessed after 72 h by the MTT assay. As shown in Figure 1, in the presence, but not in absence of IFN-α, the α-syn-induced SH-SY5Y neuroblastoma cells death and the mutant forms of α-syn were more potent than the wild-type form for this effect.

To determine the inflammatory effects of the combination of α-syn and IFN-α on primary cells, we investigated the effects of these agents on human microglial cells derived from surgical specimens. These cells were used at a fixed three times lower concentration than the THP-1 cells in Fig. 1. Shown in Figure 4 from left to right, is the source of media that were transferred to SH-SY5Y cells: 1, fresh medium; 2, α-syn treated microglial 3, α-syn + IFN-α stimulated (10 μg/ml), 4 = IFN-α (100 μg/ml); 5 = α-syn (10 μg/ml), 6 = α-syn (10 μg/ml) + IFN-α (100 μg/ml). The conditioned medium from 4 α-syn + IFN-α treated microglial cells produced the most marked reduction in the viability of SH-SY5Y cells as assessed after 72 h period by MTT (A) and LCTH (B) assays.

**Conclusions**

1. α-Synuclein actively stimulates human microglia and deleterious effects in cells in vitro.
2. This activation causes secretion of the inflammatory cytokines IL-1α and TNF-α. Mutant forms are more powerful than the wild type.
3. The secretions are also toxic to neuronal SH-SY5Y cells.
4. Knock-out case activation pathways are robustly stimulated by α-synuclein and time course studies indicate a receptor-mediated activation.
5. The main pathways stimulated are p38 MAP kinase, JNK and ERK1/2. Cell cycle progression genes are inhibited.
6. In Parkinson’s disease and other synucleinopathies, α-synuclein released from normal or damaged cells may activate glial cells and contribute to the pathology.

The initial kinetics analysis revealed α-syn-mediated increased phosphorylation of the MAP kinase ERK1/2. JNK and p38, and reduced phosphorylation of proteins involved in cytokine-dependent kinase activation and cell cycle progression. The time-course studies shown in Figure 6 demonstrate that these changes were evident as early as 10 min after exposure to α-syn. The relatively rapid rate of these responses to α-syn are consistent with a receptor-mediated activation of the MAP kinase signalling pathways. This raises the exciting possibility that microglial cells feature a receptor for α-syn that triggers their cytokine activation and which may be antagonized by therapeutic drugs.