Alpha-synuclein activates stress signalling pathways and induces neurotoxic responses in human THP-1 and microglial cells

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Abstract

Inflammation contributes to such neurodegenerative disorders as Alzheimer's and Parkinson's diseases. Activated microglial cells surround the senile plaques and Lewy bodies. We showed that human alpha-synuclein, which is a major constituent of Lewy bodies, in combination with interferon (IFN)-gamma induced an inflammatory state in human THP-1 monocytic cells. Secretions from these stimulated cells showed increased IL-1beta and TNF-alpha and were toxic towards SH-SY5Y neuroblastoma cells. Furthermore the A30P, E46K and A53T mutations of alpha-synuclein, which cause familial forms of Parkinson's disease, were more potent than normal alpha-synuclein. Human microglial cells obtained from post-surgical tissues also became neurotoxic in response to alpha-synuclein plus IFN- gamma. To investigate the signalling mechanisms evoked by alpha-synuclein treatment of THP-1 cells, profiling with antibodies for over 100 key regulatory phospho-sites was undertaken. At least 81 target phospho-sites were detected. Two-fold to 18-fold increases in activating phosphorylations of p38 MAP kinase [T180+Y182], JNK MAP kinase [T183+Y185], c-Jun [S73], RSK1 [S221 +S380], ribosomal S6 protein [S235], Hsp27 [S15], Rad17 [S645] and PKR [T451] were induced. In parallel, the phosphorylations of many proliferation-associated proteins, including B23/nucleophosmin [S4, S199, T234, T237] and Rb [S780, S807, S811, T821, T826], were reduced by greater than 50%. These findings demonstrate that alpha-synuclein acts as a potent stimulus of microglial cells and could explain the increased toxicity of mutant forms of alpha-synuclein. Inhibition of microglial activation by alpha-synuclein could be beneficial in the treatment of Parkinson's disease.

Introduction

The physiological functions of α-synuclein (α-syn) have not been explored fully. Much attention has been focused on its possible involvement in neurodegeneration since mutations are a causative factor in Parkinson's disease (PD). Currently there are three known point mutations A30P, E46K and A53T that result in PD. Aggregated α-syn is a major component of Lewy bodies, which are hallmarks of PD and Lewy body dementia. The cellular and molecular mechanisms underlying the pathological action of α-syn are currently 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 glial cells. Significant amounts of α-syn are secreted from cells under physiological conditions. Accumulation of extracellular α-syn due to leakage from damaged cells or as a result of secretion might have pathological consequences. We report here that the disease-causing mutations of α-syn A30P, E46K and A53T are more potent than normal α-syn in inducing human THP-1 cell neurotoxicity and secretion of two pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Profiling with antibodies for over 100 key regulatory protein phospho-sites revealed that α-syn stimulates the p38, JNK and ERK1/2 mitogen-activated protein (MAP) kinase pathways in a receptor-mediated fashion. By contrast, cell cycle signalling pathways involving cyclin-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-γ. THP-1 cells were incubated with various concentrations (shown on the abscissas of Figure 1) of α-syn in the absence (A) or presence (B) of 150 U ml^{-1} IFN-γ for 24 h. The wild-type as well as A30P, E46K and A53T mutated forms of α-syn were used without aggregation. Subsequently the cell-free supernatants of THP-1 cells were transferred to the wells containing 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-γ, all of the α-syn species induced SH-SY5Y neuroblastoma cells death, and the mutant forms of α-syn were more potent than the wild-type form for this effect.

Figure 1.

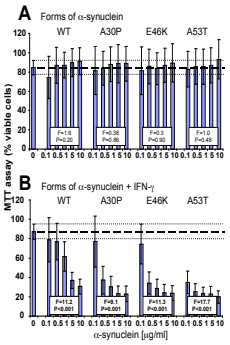
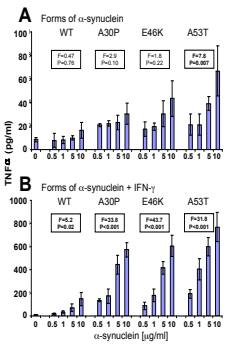


Figure 2.



We next assessed the effects of α-syn on TNF-α and IL1-β secretion by THP-1 cells. α-Syn or its mutated forms were administered to THP-1 cells in the absence (A) or presence (B) of 150 U ml^{-1} IFN-γ. The TNF-α (Figure 2) and IL1-β (Figure 3) concentrations in cell-free supernatants were measured 48 h later. All of the α-syn species induced TNF-α and IL1-β secretion, and the mutant forms of α-syn were more potent than the wild-type form, and these effects were strongly potentiated by the treatment with IFN-γ.

Figure 3.

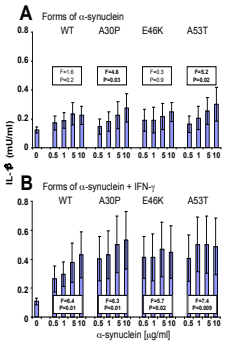
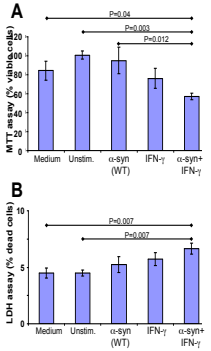
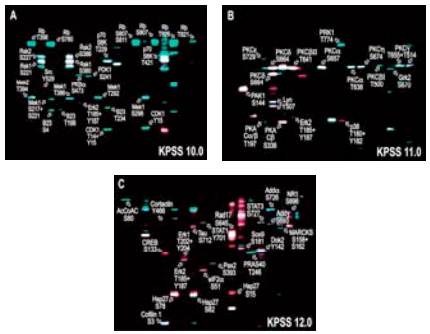


Figure 4.



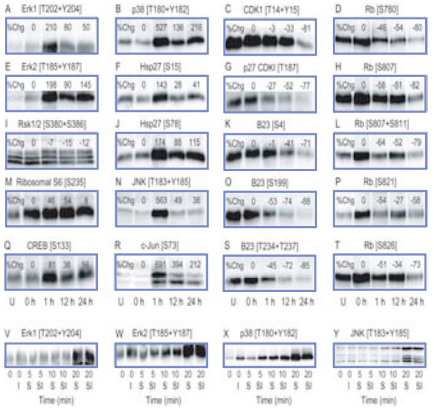
To determine the immunomodulatory effects of the combination of α-syn and IFN-γ on primary cells, we investigated the effects of these agents on human microglia cells derived from surgical specimens. These cells were used at a five times lower concentration than the THP-1 cells in Fig. 1. Shown in Figure 4 from left to right, are the sources of media that were transferred to SH-SY5Y cells: 1 - fresh medium; 2 – unstimulated microglia; 3 – microglia stimulated with α-syn (10 μg ml^{-1}); 4 – IFN-γ (150 U ml^{-1}); and 5 – α-syn (10 μg ml^{-1}) plus IFN-γ (150 U ml^{-1}). The conditioned medium from 24 h α-syn and IFN-γ treated microglia cells produced the most marked reduction in the viability of SH-SY5Y cells as assessed after 72 h period by MTT (A) and LDH (B) assays.

Figure 5.



To investigate the signalling mechanisms by which α-syn induced secretion of TNF-α and IL1-β in THP-1 cells, we performed Kinetworks™ KPSS 10.0, 11.0 and 12.0 phospho-site analyses with the Kinexus muti-immunoblotting services. These three screens utilize 108 different phospho-site-specific antibodies for detection of numerous signal transduction proteins. Each panel of Figure 5 shows an overlay of two ECL blot images of lysates from cells: 1, treated for 24 h with IFN-γ alone (150 U ml^{-1}); and 2, treated with IFN-γ plus α-syn (10 μg ml^{-1}). Bands that look red, green and white showed increased, decreased and unchanged phosphorylation, respectively, in sample 2 compared to sample 1.

Figure 6.



In panels A to T, "U" corresponds to lysates from THP-1 cells that were not treated with IFN-γ, and the other lanes correspond to lysates prepared from cells that were treated with α-syn plus IFN-γ for 0 to 24 h. In panels V to Y, "I" corresponds to treatment with IFN-γ for 24 h, and with α-syn (S) for an additional 5 to 20 min.

Conclusions

1. α-Synuclein actively stimulates human microglia and microglia-like THP-1 cells *in vitro*.
2. This activation causes secretion of the inflammatory cytokines IL-1β and TNF-α. Mutant forms are more powerful than the wild type.
3. The secretions are also toxic to neuronal SH-SY5Y cells.
4. Known T-cell 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 kinases 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 Kinetworks™ analysis revealed α-syn-mediated increased phosphorylation of the MAP kinases ERK1/2, JNK and p38, and reduced phosphorylation of proteins involved in cyclin-dependent kinase action and cell cycle progression. The time course studies shown in Figure 6 demonstrate that many of 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 cytotoxic activation and which may be antagonized by therapeutic drugs.

Figure 7.

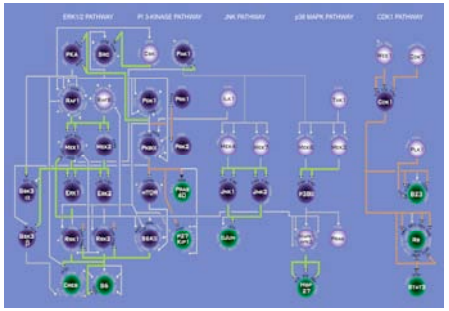


Figure 7 depicts an overlay of many of the major α-syn-induced changes in five important signalling pathways in THP-1 cells that were detected with the Kinetworks™ KPSS 10.0, 11.0 and 12.0 phospho-site screens. Actual proteins and phospho-sites tracked appear in purple and black, respectively. α-syn associated increases in protein phosphorylation are shown with solid green arrows and decreases are indicated with dashed orange arrows. These phosphorylation changes caused by α-syn in the presence of IFN-γ are characteristic of the actions of cytokines that activate THP-1 cells.