# **ImmunoTools** *special* Award 2014

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## Neuroinflammation in Parkinson's disease: role of LRRK2 mutation.

Parkinson´s disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons of the nigrostriatal system, causing the motor symptoms observed in these patients (1; 2). Although it is not well understood, the pathogenesis of PD has been linked to genetic factors as well as environmental factors, such us inflammation, in which microglial activation in the nigrostriatal system is ubiquitously found (3).

Several works have reported that a mutation on LRRK2 gene (LRRK2-G2019S) has been identified in sporadic and familial PD cases. LRRK2 gene encodes an enzyme called *leucine-rich repeat kinase 2* and now days its function is not fully understood. LRRK2 was found to be highly expressed in B cells, monocytes, and dendritic cells (4, 5). Also, LRRK2 expression was induced by Interferon (IFN)-gamma and LPS in a human macrophage cell line, in human CD11b-positive monocytes and primary mouse and rat microglia (4-6). In addition, there are results that show that cells with mutant LRRK2 are more vulnerable to cell death.

These evidences suggest that LRRK2 play a relevant role in PD development and inflammatory response (7, 8).

The general aim of our laboratory is to provide information to design protective or regenerative therapies for PD in order to find effective therapies and methods to halt disease progression. To reach this goal, we have generated 5 animal models of neurodegeneration, the technology of cellular reprogramming as a source of dopaminergic neurons from induced pluripotent stem cells (iPS) and *in vitro* models of PD (9, 10, 11). iPS cells are generated by reprogramming adult fibroblasts back to an immature, pluripotent state (11). This technology can be used for disease modeling.

Because LRRK2 mutation, closed related to PD and inflammation, is thought to be involved in the pathogenesis of the disease, we propose to study the functional role of LRRK2 on neurodegeneration mediated by inflammation. This project will combine different approaches, *including in vitro* and *in vivo* studies, cellular imaging, molecular biology and immunochemistry.

*In vitro* assays: iPS cells from PD patients with LRRK mutation (LRRK2mut) and healthy donors (age and gender matched) will be used to study differentiation process to dopaminergic neurons in order to analyze the role of this gene on cellular differentiation. These cells will be used in co-culture assays with human monocytes in order to study pro and anti-inflammatory cytokines profiles. Also, conditioned media from activated or naïve monocytes will be used on dopaminergic neurons in order to analyze cell death. The results from *in vitro* models will give us information about the relation between LRRK2 mutation and inflammation.

*In vivo* models: Rats injected with adenoviral vectors expressing mutated LRRK2 or control adenovectors will be used as an animal model for PD (12-16). Microglial activation and lymphocytes infiltration will be analyzed in order to know if neuroinflammation is increased in animals with LRRK2 mutation.

The study of LRRK2 role on the inflammatory response has a significant potential for a better understanding of the impact of this protein on inflammation and PD pathogenesis.

For the iPS cultures and in vitro experiments, we will used the following recombinant human cytokines from ImmunoTools: rh Activin A; rh BDNF; rh beta NGF; rh BMP-2; rh EGF; rh GDNF; rh SHH; rh TGF-beta3; rh IL-1alpha / IL-1F1; rh IL-1beta /IL-1F2; rh IL-10.

Analysis of in vitro neuroinflammation induced by LRRK2mut will be carry out using: Anti-human antibodies for flow cytometry: FITC-CD11b; PE-CD19, PE-CD14; FITC-CD8 and PE-CD4 and Human ELISA-set for: human IL-4, human IL-6, human IL-8, human TNF-a.

For *in vivo* analysis of inflammatory response, we are going to use ImmunoTools recombinant rat cytokines: rr IFNgamma; rr IL-1beta; rr IL-10; rr TNFα

References


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