the functional consequences of this activation using human samples and complement blockers to test complement as a therapeutic target.

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### Complement drives Parkinson's disease neuropathology through activation of microglial C5a receptors

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder and imposes a severe social and economic burden on ageing populations. PD results from the progressive loss of dopaminergic neurons which is accompanied by a chronic neuroinflammatory response that propagates disease progression. Despite clear evidence for complement involvement in Alzheimer's disease, the contribution of complement to PD neuropathology remains poorly defined. Using publically available data, we demonstrate that complement is widely upregulated in PD patient brains, at sites of dopaminergic neuron loss, and in peripheral blood. Similarly, activation of complement is observed in multiple preclinical PD models. Genetic deletion of key complement effectors at the level of C3, C5, and MAC highlighted a critical role for complement C5a receptors (C5aR1) in driving neurodegeneration in vivo in response to dopaminergic toxins. Fibrillar α-synuclein aggregates (PFF-synuclein), the predominant protein found in PD brain Lewy bodies, directly activated complement to generate C5a and markedly increased C5aR1 expression in human and mouse microglia. Oral administration of a C5aR1 antagonist significantly protected against behavioral motor deficits and nigrostriatal dopaminergic degeneration in acute (28-day 6-OHDA) and chronic (12-month PFF-synuclein) mouse models of PD. Notably, delaying drug administration until symptom onset prevented further motor functional decline and remained neuroprotective. Live visualisation of neuroinflammation using [18F]DPA-714 PET/CT-imaging, demonstrated that both prophylactic and therapeutic inhibition of C5aR1 blunted microglial activation in living mice. Mechanistically, cell-intrinsic microglial NLRP3 inflammasome activation by multiple stimuli was impaired in the genetic absence of C5aR1. Furthermore primary human microglia were unable to secrete IL-1β in response to α-synuclein fibrils in the presence of C5aR1 inhibitors. Targeting this complement-microglia-inflammasome axis with brain-permeable inhibitors could be a feasible approach to tame neuroinflammation, and slow neuronal death in people living with PD.

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### Leptospira interrogans leptolysin displays proteolytic activity against complement proteins

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Pathogenic Leptospira species are extremely efficient in disseminating in the host, a fact attributed to their ability to escape complement system activation, and to degrade extracellular matrix and other components of the human plasma. Recently, our group evaluated the proteolytic activity of secreted proteins by leptospires, and exoproteome analyzes of these bacteria allowed the identification of some proteases, including the metalloprotease pappalysin-1 domain protein, which we named leptolysin. In this work we produced and functionally characterized leptolysin from L. interrogans to expand our knowledge on this metalloprotease from Leptospira in the processes of invasion and immune evasion. According to in silico analyzes this protease belongs to the category of short pappalysins, also found in other bacteria. Leptolysin is present in all Leptospira species but is more conserved among pathogenic species of the P1 subclade. A preliminary biochemical characterization of its proteolytic activity was performed using FRET (Free Resonance Energy Transfer) peptides. The enzyme exhibited maximum activity at pH 8.0 and 37°C, was active in the presence of different salts and was strongly inhibited by EDTA and 1,10-phenanthroline. It showed a marked preference for arginine residues in the P1 position. The proteolytic activity of recombinant leptolysin on host molecules was also evaluated in vitro and in vivo. The metalloprotease was active against extracellular matrix proteins (proteoglycans and fibronectin), coagulation cascade molecules (fibrinogen and thrombin) and effector proteins of the human complement system (C2 to C9). A leptolysin knockout strain (Δlic13434) was produced and characterized. This strain showed lower survival in normal human serum (SHN) compared to the wild-type strain. However, in a model of epicutaneous infection in hamsters, no attenuation of virulence was observed with the knockout strain, although the bacterial load in the kidneys of these animals was lower than that observed in animals inoculated with the wild-type strain. Finally, data with sera from leptospirosis patients suggest that leptolysin is produced during natural infections by pathogenic leptospires. The characterization of toxins, their targets and mechanisms of action can help in the development of strategies to combat leptospirosis.

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