Editorial

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Alpha-Synuclein to the Rescue: Immune Cell Recruitment by Alpha-Synuclein during Gastrointestinal Infection

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Abstract
Intraneuronal accumulation of misfolded alpha-synuclein in the central and peripheral nervous systems is strongly linked to Parkinson disease (PD) and other related synucleinopathies. In rare inherited forms of PD, point mutations or gene multiplications mediate the formation of alpha-synuclein protein aggregates. However, in most PD cases it is presumed that the combined effects of ageing and environmental factors drive the formation of alpha-synuclein aggregates. Despite advances regarding alpha-synuclein pathobiology, the normal functions of this protein and factors that regulate its expression are not well understood. We discuss a recent study reporting that viral infection induces alpha-synuclein expression in neurons of the gastrointestinal tract. Alpha-synuclein levels increased during norovirus infection in the duodenum of children. In an in vitro paradigm, monomeric and oligomeric alpha-synuclein acted as chemoattractants for neutrophils and monocytes, and promoted the maturation of dendritic cells. This suggests that alpha-synuclein facilitates immune responses to infection. We explore the possibility that intestinal infections, and associated inflammation, place individuals at increased risk of PD by increasing alpha-synuclein levels and promoting the formation of alpha-synuclein aggregates that propagate in a prion-like fashion via the vagal nerve to the brainstem.

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A recent study by Stolzenberg et al. [1] found that a common infection in the human gastrointestinal (GI) tract results in an upregulation of alpha-synuclein in enteric neurons, which may serve to rally an immune response to combat pathogens. Intraneuronal accumulation of misfolded alpha-synuclein is central to the pathogenesis of Parkinson disease (PD) and related synucleinopathies, and is promoted by high levels of alpha-synuclein. Genetic studies have shown that an increased gene dosage of alpha-synuclein, due to duplications or triplications of the alpha-synuclein locus, leads to autosomal dominant forms of a PD-like condition [2, 3]. Small increases in the levels of alpha-synuclein mRNA and protein, coupled to single nucleotide polymorphisms near the gene locus, are associated with risk for sporadic PD [4, 5]. Consequently, elevated levels of intraneuronal alpha-synuclein may pose a risk for the development of PD.

Alpha-synuclein protein aggregates represent a major component of Lewy bodies and Lewy neurites, the pathological hallmark of PD and related synucleinopathies, including dementia with Lewy bodies and multiple system atrophy [6, 7]. In the PD brain, Braak and colleagues [8, 9] originally suggested that neuronal alpha-synuclein aggregates develop gradually, and progressively involve brain structures that are part of 2 anatomical systems. In one proposed model, Lewy pathology first affects peripheral nerves innervating visceral organs (e.g., the heart and gut) and then sequentially involves brain regions along a caudorostral axis, starting in the lower brainstem (including the dorsal motor nucleus of the vagus nerve in the medulla). The other scenario starts with Lewy aggregates in the olfactory bulb and these then appear in other olfactory nuclei and interconnected brain regions. Regardless of whether the trigger site is in the gut, olfactory system, or both, it is thought that the substantia nigra is affected years later, and the result is degeneration of the dopaminergic nigrostriatal pathway, which gives rise to the classic motor symptoms [10]. In advanced PD, Lewy pathology is also evident in numerous forebrain structures, including the cerebral cortex, which might underlie cognitive and psychiatric symptoms [8, 11].

In addition to the motor symptoms, PD patients commonly exhibit many nonmotor manifestations, including dysfunction of the GI tract (typically constipation) and olfactory impairment, both of which often predate the onset of motor symptoms by as many as 20 years [12, 13]. Furthermore, alpha-synuclein aggregates are evident in the enteric nervous system of PD patients [14]. It is suggested that alpha-synuclein pathology in enteric neurons develops early in the disease process, before motor symptom onset, i.e., during what is classified as "prodromal PD" [15, 16]. Numerous experimental studies have shown that alpha-synuclein can spread in a prion-like fashion from cell to cell, triggering the formation of Lewy-like aggregates in neurons [17, 18]. Because alpha-synuclein aggregates can ascend via the vagal nerve in experimental models [19], it has been suggested that the GI tract is an important site of origin of PD [9, 20]. Furthermore, epidemiological studies report that prior truncal vagotomy is coupled to reduced PD risk [21, 22]. This suggests that alpha-synuclein aggregates originating in the enteric neurons of the GI tract may travel up the vagal nerve to seed and spread the pathology in the brain.

Despite its importance in PD pathogenesis and progression, the normal function(s) of alpha-synuclein remain unclear. Current thinking is focused on a possible role in vesicle recycling and neuronal synaptic transmission [23]. On the other hand, an emerging school of thought suggests that alpha-synuclein plays a role in the response to infections. Alpha-synuclein expression increases in the brain in response to West Nile virus and Venezuelan equine encephalitis, and the absence of alpha-synuclein (in knockout mice) exacerbates the viral encephalitis [24, 25]. Furthermore, gut infections by *Escherichia coli* that produce the amyloid protein curli promote alpha-synuclein aggregation both in the gut and brain [26].

In a recently published study by Stolzenberg et al. [1] in the *Journal of Innate Immunity*, the authors added support to the view that alpha-synuclein might be involved in the innate immunity response. They examined alpha-synuclein levels in biopsies of children with duodenal inflammation and of intestinal transplant recipients that had contracted norovirus, a common GI tract pathogen. The levels of alpha-synuclein in the gut mucosa were found to correlate with the degree of infiltration of immune cells in the duodenum of children (mean age of 12 years). The source of inflammation was primarily *Helicobacter pylori*, which is estimated to be present in the GI tract of 50% of people [27]. Hence, a common pathogen can increase alpha-synuclein levels even at a young age. In individuals that received an intestinal transplant, norovirus virus infection induced a persistent elevation in alpha-synuclein, even 6 months after the clinical resolution of the infection. Given the high prevalence of these pathogens, it is surprising that so little alpha-synuclein has been detected in the mucosa of the small intestine in most (but not all [28]) previous studies [14, 29, 30]. A possible explanation for the majority of earlier studies dem-

onstrating little alpha-synuclein in the small intestine may be related to the intestinal regions examined, and it would be interesting to determine whether certain intestinal subregions are more prone to infection-induced alpha-synuclein upregulation.

Why do intraneuronal alpha-synuclein levels increase following GI tract infection? In vitro studies performed by Stolzenberg et al. [1] showed that human alpha-synuclein conformations (monomeric and oligomeric alpha-synuclein, plus acetylated N-terminus fragment) were chemotactic, and promoted the migration of neutrophils and monocytes towards chamber compartments containing increasing concentrations of human alpha-synuclein. Alpha-synuclein was also a maturation factor for human dendritic cells, which are antigen-presenting cells of the immune system. This points to a possible role of extracellular alpha-synuclein in mobilizing immune defenses against pathogens. As we mentioned above, given the potential for the prion-like spread of alpha-synuclein to the brain, robust or prolonged immune responses in the GI tract may contribute to an increased risk of developing PD.

Activation of the immune cells, particularly microglia, is intricately linked to the degeneration of neurons in the PD brain [31–33]. Notably, there are numerous reciprocal links between immune cell activation and alpha-synuclein aggregation. For example, alpha-synuclein fragments activate immune cells in various PD models [34], and are suggested to be one of the triggers for microglial activation in the brains of PD patients [35]. The GI tract of PD patients has elevated numbers of proinflammatory microbes, and these have been suggested to promote microglial activation and alpha-synuclein aggregation in the brain via microbial metabolites (short-chain fatty acid signaling) [36]. In aged animals, activated macrophages surround and invade enteric ganglia that express increased amounts of alpha-synuclein [37]. The levels of mRNA of inflammatory cytokines (i.e., TNF-α, IL-1β, IL-6, IFN-γ, Sox10) are elevated in colonic biopsies from PD patients, in a manner that correlates with disease duration [38]. Together, these findings indicate that increased expression of neuronal alpha-synuclein and immune system activation participate in a self-reinforcing cycle, which for some individuals can become a vicious and pathogenic cycle. This process can involve the release of alpha-synuclein to the extracellular space, potentially leading to the propagation of Lewy pathology between the peripheral and central nervous system, and eventually between brain regions.

The majority of individuals are exposed to numerous pathogens and infections throughout life. The study by Stolzenberg et al. [1] suggests that such pathogens cause an upregulation of endogenous alpha-synuclein in the nerves innervating the gut, and possibly some of this is released in order to stimulate an innate immune response that protects against the pathogens. Due to the proposed triggering of an increase in neuronal and extracellular alpha-synuclein, infections with certain gut pathogens can constitute a risk factor for PD. A similar reasoning might apply to the olfactory system, which is exposed to numerous pathogens via the olfactory epithelium and is suggested to be one of the first sites of alpha-synuclein aggregation in PD [39]. This raises the possibility that PD is not the result of a select causative agent(s), but rather a failure in the systems in place that should protect against alpha-synuclein aggregation. As such the key question becomes: "what prevents the majority of individuals from developing PD?" If excessive alpha-synuclein levels and aggregation caused by relatively common transient or chronic gut infections always prompted the propagation of aggregates to the brainstem via the vagal nerve, then the frequency of PD would be much greater. Hence, in most individuals, alpha-synuclein aggregates must be cleared after the pathogen exposure. One can speculate that in some individuals these protective factors erode with age, an effect further amplified by genetic risk, and in rare cases eventually lead to PD. If the endogenous function of alpha-synuclein is indeed to trigger an immune response with the consequence that continued alpha-synuclein elevation poses a PD risk, it will be important to understand what mechanisms are responsible for modulating pathogen-induced alpha-synuclein increases in the gut. These protective biological pathways could explain intestinal accumulations of PD pathology, and could be useful targets for PD treatment and preventative measures.

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