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The vermiform appendix in Parkinson's disease: At the crossroad of peripheral immunity, the nervous system and the intestinal microbiome

The dual-hit hypothesis of Parkinson's disease (PD) pathogenesis, suggesting that pathological aggregation of α-synuclein (α-syn) may originate in the enteric nervous system, led to identify the vermiform appendix as the gastrointestinal region richest in α-syn [1]. As a consequence, many studies thereafter focused on the potential relationship between appendix (and appendectomy) and PD. Mendes et al. [2] studied 295 PD patients concluding that appendectomy might delay PD onset, even if only among patients with late onset. Studies on larger populations however found no protective effects of appendectomy on risk of PD, and in some cases even suggested a moderate but nevertheless significantly increased risk [3–5]. Recently, Killinger et al. [6] published the so far largest epidemiological study on PD and the appendix, providing renewed support to the hypothesis of a protective role of appendectomy. By use of the Swedish National Patient Registry, the authors found that over 30 years the incidence of PD was about 19% less among individuals who had an appendectomy, and that those appendectomized at least 30 years before PD onset had disease onset 3.6 years later. Authors subsequently analysed the Parkinson's Progression Markers Initiative data sets, finding that PD patients who underwent appendectomy at least 20 years before diagnosis received their diagnosis about 1.6 years later than patients without appendectomy. However, despite the large sample size (nearly 1,7 million individuals), even this study has been criticized due to the observational design. Moreover, stratified analysis showed protective effects of appendectomy only in rural populations (but not in urban groups), suggesting a role for uncontrolled additional factors [7].

The appendix has been considered for long time an evolutionary redundancy, however several lines of evidence now increasingly point to its key immunological functions. The appendix is indeed rich in lymphoid tissue and contains large amounts of immune cells. Moreover, it hosts biofilms of commensal bacteria which continuously shed into the intestinal lumen [8]. The appendix mucosa hosts large amounts of macrophages as well as of T and B lymphocytes, including regulatory T lymphocytes, and it is an area of antigen presentation and immune stimulation. The dome epithelium of the mucosa is located above the lymphoid follicles, which extend from the submucosa into the lamina propria, and contains B and T lymphocytes, macrophages, and follicle dendritic cells, exerting key antigen presentation functions. T cell areas at the bottom of follicles contain macrophages and T cells, predominantly of the CD4+ lineage [9]. Thus, also considering its extensive innervation, the appendix might well represent a key crossroad connecting the immune system, the nervous system and the intestinal microbiota, with potentially huge implications for PD pathogenesis (Fig. 1).

Indeed, neuroinflammation supported by peripheral inflammation and autoimmunity has been identified as critical in PD pathogenesis [10], and recently Sulzer et al. [11] reported that α-syn-derived peptides may act as antigenic epitopes driving helper and cytotoxic T cell responses in PD patients. This observation, together with the finding by Gray et al. [1] that α-syn in the appendix is contained also in mucosal macrophages, may well imply the occurrence of local presentation of a-syn-related antigens to T lymphocytes. The study by Killinger et al. [6] further strengthens this hypothesis by showing that healthy appendix tissues contain potentially pathogenic α-syn varieties, and that full-length human α-syn added *in vitro* to appendix tissue lysates undergoes cleavage, resulting in aggregated and truncated forms. The potential of such pathogenic α-syn varieties and modified forms to act as triggers for peripheral immunity deserves careful consideration.

Evidence from animal models indicates that the appendix plays a role in oral tolerance, promoting in Peyer's patches a reduction in Th1 cells thus shifting from proinflammatory Th1 to antiinflammatory Th2 responses during oral tolerance induction, and that appendectomy suppresses such mechanisms [12]. Circumstantial evidence in humans suggests that acute appendicitis is characterized by an uncontrolled Th1 response, resulting in increased production of proinflammatory cytokines such as interferon (IFN)-γ [13]. We recently reported that peripheral adaptive immunity in PD patients is characterized by a complex Th1 bias including preferential differentiation of naïve CD4+ T cells towards the Th1 lineage, and increased production of IFN-γ and tumor necrosis factor-α by CD4+ T cells which is resistant to CD4+ T regulatory cells-mediated inhibition. This Th1-biased immune signature occurs in both drug-naïve patients at PD diagnosis as well as in patients on dopaminergic drugs, suggesting that it is a very early phenomenon accompanying (or even possibly preceding) disease onset [14]. There is so far no direct evidence for the origin of such peripheral Th1 bias in PD patients, however it is remarkable that a recent study, comparing the fecal microbiomes of PD patients and controls, reported in feces of PD patients nearly 80% decreased abundance of *Prevotellaceae* and increased abundance of other families including *Lactobacillaceae*, a bacterial strain inducing Th1-type systemic immune responses [15].

Taking into account all such heterogeneous lines of evidence, it might be speculated that a key trigger to PD pathogenesis is represented by antigenic recognition of pathogenic α-syn forms by T lymphocytes in the appendix (and possibly in general in gut-associated lymphoid tissue), in the context of a Th1-biased environment, either constitutive or induced by inflammatory processes such as appendicitis. Indeed, it has been already proposed that PD may start in the gut, where α-*syn*-related neurodegeneration of enteric neurons is frequent and early during PD [16]. Presentation of α-syn to lymphocytes homing into the gut-associated lymphoid tissue, in the context of a disbiotic intestinal milieu, might well promote α-syn antigenic potential, driving detrimental T cell responses in PD patients [11], and activated α-*syn*-specific T cells might thereafter migrate to the brain favoring subsequent neuroinflammation, finally leading to neurodegeneration [17]. Would the appendix contibute to induction of tolerance to antigens also in humans, it could be additionally speculated that local mechanisms might

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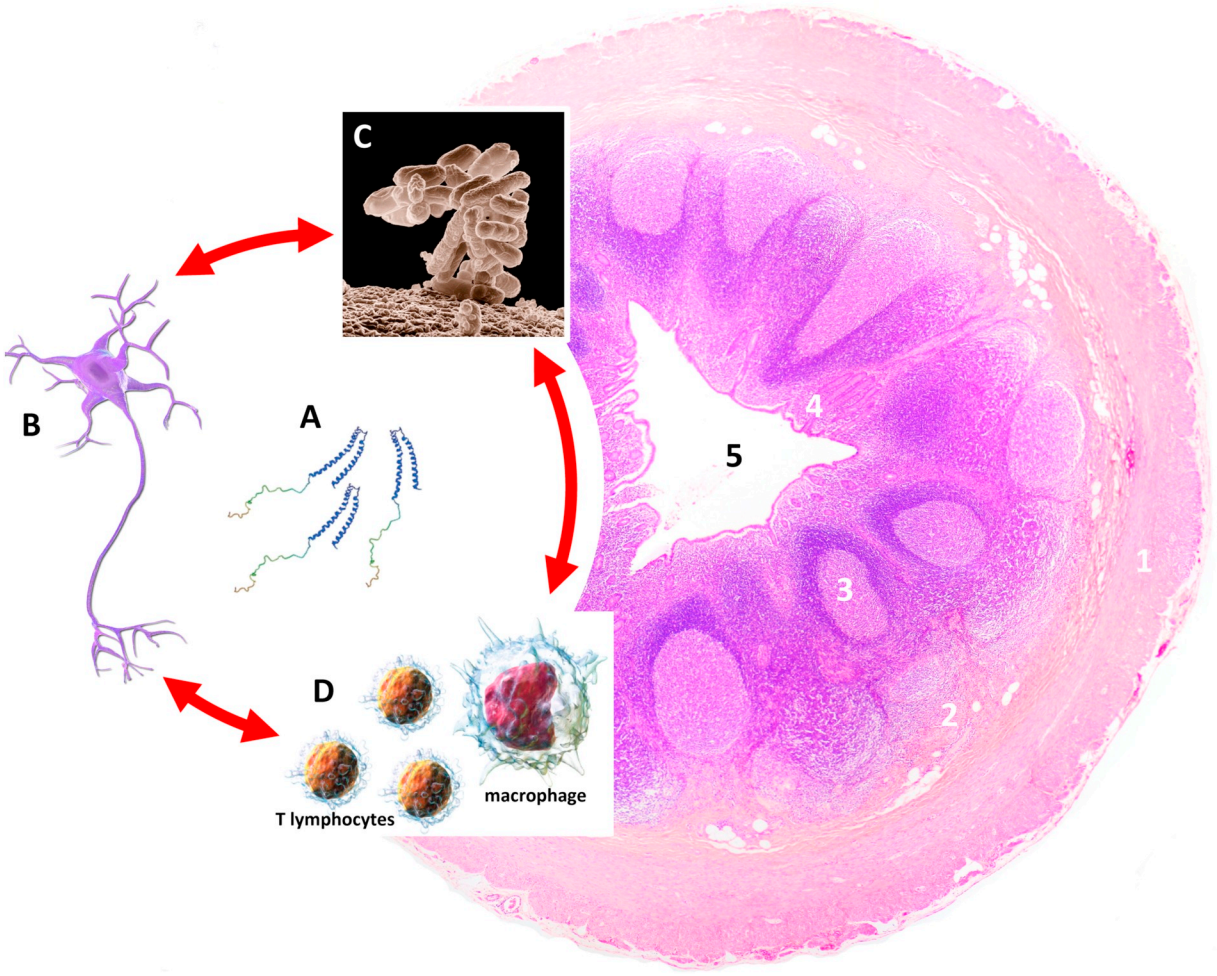

Fig. 1. The appendix as a crossroad connecting the immune system, the nervous system and the intestinal microbiome in PD. Alpha-synuclein (α-syn, A) occurs at high levels in the vermiform appendix, and α-*syn*-related degeneration of enteric neurons (B) is a frequent and early manifestation of PD. The gut microbiome (C) may contribute to local biotransformation of α-syn to pathogenic and antigenic forms, in turn recognized by resident immune cells (D), including macrophages which process and present α-syn antigens to T lymphocytes. Whether α-syn recognition by the immune system results in induction of tolerance or in an autoimmune inflammatory response, subsequently spreading to the brain, may well result from the complex interaction among intestinal microbiome, the enteric nervous system and the immune system. The figure background shows a transverse section of a healthy adult appendix: 1, muscularis externa; 2, submucosa; 3, lymphoid follicle; 4, mucosa and 5, lumen (background figure is courtesy of Professor William L. Todt, Concordia College (MN, http://faculty.cord.edu/todt), while other parts of the figure are from the Wikimedia Commons - http://commons.wikimedia.org).

even drive α-*syn*-specific T cells towards tolerance or cytotoxicity depending on the microenvironment. Remarkably, during appendicitis the inflammatory infiltrate is constituted mainly by neutrophils while in more chronic stages lymphocytes also occur, and studies in rodents show increased CD4 + and CD8 + T cells and higher amounts of CD4 + T regulatory cells, which however do not occur in elderly animals and in the presence of antimicrobials such as antibiotics (reviewed in 9).

Confounding factors possibly underlying the so far conflicting and inconclusive results regarding the relationship between appendix and PD could therefore include the composition of intestinal microbiome, the local and systemic immune environment as well as the interplay with enteric nerves. Future studies should possibly focus on appendicitis rather than simply on appendectomy, which is just a proxy of inflammatory processes occurring at this level. Indeed, appendicitis not always results in surgical appendectomy, medical treatment with antibiotics representing in many instances a potential noninvasive alternative. Other critical factors include: the stage of appendicitis, either acute of chronic, the use of antibiotics and their ability to affect gut microbiome, the individual local and systemic immune profile also in relation to age. Different intestinal acute inflammatory conditions should be in addition taken into account, like for example diverticulitis. There is moreover strong need for better characterization of the complex relationship between the immune system and the intestinal microbiome, and of their respective roles and contributions to the eventual local conversion of native α-syn to pathogenic forms with subsequent recognition by immune cells as neoantigens. Thorough understanding of such intricate processes will be difficult, but nevertheless will also likely shed new light on the still elusive early pathogenesis of PD, possibly providing novel opportunities for disease-modifying therapies.

Meanwhile, it might be relevant also to note that the first paper to describe acute appendicitis was read in 1812 in London by James Parkinson, on behalf of his son John, to the Medical and Chirurgical Society, about five years before the publication of his classic *Essay on the shaking palsy*, which established for the first time 'paralysis agitans' as a clinical entity, later named by Jean-Martin Charcot as 'maladie de Parkinson' [18]. Actually, the relationship between appendix and PD looks much earlier and deeper than anyone might have possibly anticipated.

Author contributions

All the authors were equally involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. All authors agree to be accountable for all aspects of the article.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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