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Is there a role for *Mycobacterium avium* subspecies *paratuberculosis* in Parkinson's disease?

Giannina Arru [a,b,1], Elisa Caggiu [a,1], Kai Paulus [b], Gian Pietro Sechi [b], Giuseppe Mameli [a], Leonardo A. Sechi [a,*]

[a] Dipartimento di Scienze Biomediche, Sezione di Microbiologia e Virologia, Università di Sassari, Italy
[b] Dipartimento di Medicina Clinica e Sperimentale, Università di Sassari, Viale San Pietro 43b, 07100 Sassari, Italy

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ABSTRACT

In Parkinson's disease (PD) ZnT proteins play an important role. Zinc is a co-factor of numerous enzymes and stabilizes the tertiary structure of several proteins. Nothing is known about previous infections mediated by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). We evaluated if a previous infection with MAP could induce the production of antibodies that cross-reacted with the Znt homologous antigenic peptides associated to Parkinson. The humoral response toward MAP3865c peptides, ZnT3 and ZnT10 was evaluated. The hypothesis of cross-reactivity needs to be confirmed; we have observed the presence of MAP in PD patients by PCR, positivity to MAP3865c peptides, therefore MAP infection but not cross-reaction with human homologous Znt proteins.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the formation of Lewy bodies (LBs) (Spillantini et al., 1997; Lee and Trojanowski, 2006). The exact mechanism of PD is still unclear. α-synuclein is the main component of the PD Lewy bodies (LBs) and one of the key proteins in the disease pathogenesis. In addition, the role of ZnT family proteins in Parkinson and Alzheimer's diseases is well documented (Prakash et al., 2015, Szewczyk, 2013; Lovell, 2009, Yokel, 2006).

Zinc is one of the most prevalent trace elements in the human body. It is a key structural component of proteins, and a co-factor of numerous enzymes that regulate cellular processes and cellular signaling pathways essential for brain and systemic physiology (Takeda, 2000). It is also important for his role in stabilizing the tertiary structure of several proteins. Zn^{2+} homeostasis mediated by ZnT3 is involved in maintenance of cognition and memory, that both can be affected by the loss of ZnT3 with consequent reduction of Zn^{2+} (Adlard et al., 2010). Several studies reported the role of Zn^{2+} in facilitating aggregation of Aβ, tau and α-synuclein proteins (Bush et al., 1994; Curtain et al., 2001; Boom et al., 2009; Mo et al., 2009; Paik et al., 1999; Yamin et al., 2003). ZnT10 has also been studied in patients with neurological disorders and Parkinsonism showing that two different homozygous frame shift mutations in ZnT10 have been implicated in these pathologies (Quadri et al., 2012). Therefore, an imbalance of zinc homeostasis could lead to the onset of neurological disorders such as depression, Parkinson's disease, Alzheimer's disease (AD), or amyotrophic lateral sclerosis (ALS) (Szewczyk, 2013). Nothing is known about previous infections mediated by *Mycobacterium avium* subsp. *paratuberculosis*, an intestinal pathogen (Sechi LA and Dow CT 2015), although it has been hypothesized a link between MAP infection and PD (Dow, 2014) and several studies have shown an homology between human ZnT8 and MAP 3865c protein that is involved in type 1 diabetes (Pinna et al., 2014; Masala et al., 2011, 2013, 2014a). Given the similarities of the ZnT family of proteins and the homologies between ZnT8 and MAP 3865c, the aim of our study was to evaluate if a previous infection with MAP could induce the production of antibodies that cross-react with the Znt homologous antigenic peptides associated to Parkinson. The humoral response toward MAP3865c peptides, ZnT3 and ZnT10 was evaluated.

2. Materials and methods

2.1. Subjects

Patients were enrolled at the Neurology Clinic of the University Hospital of Sassari, healthy controls patients were enrolled at the Blood Transfusion Centre of Sassari. Peripheral venous blood of patients and healthy control donors were collected. The diagnosis of Parkinson's (PD) was based on the established criteria (Gelb et al., 1999). The cohort included 40 PD patients (M/F = 2.33, mean age 69.83 ± 7.95) and 40

* Corresponding author at: Dipartimento di Scienze Biomediche, Università di Sassari, Viale San Pietro 43b, 07100 Sassari, Italy.
E-mail address: sechila@uniss.it (L.A. Sechi).
[1] These authors contributed equally to the manuscript.

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age-and-sex-matched healthy controls (M/F = 2.33, mean age 65.76 ± 9.60). The local ethics committee approved the study (Prot. N 2159/CE 2015, Azienda Sanitaria Locale 1, Sassari, Italy) and all blood samples were obtained with fully informed consent.

2.2. Antigens

The peptides described in Table 1 were included in the study. All of them were synthesized commercially at >90% purity (LifeTein, South Plainfield, NJ 07080, USA).

2.3. ELISA

The antibody value toward the selected peptides was evaluated by ELISA assay as previously described. (Arru et al., 2015).

2.4. VersaTREK®/ESP® Culture System II for detection of Mycobacterium avium ssp. paratuberculosis

Venous blood from PD patients was used to isolate peripheral blood mononuclear cells (PBMCs) from 10 ml of blood by density gradient centrifugation on Ficoll-Paque Plus, (GE Healthcare Bioscience, Sweden). PBMC resuspended in 1 ml of PBS were inoculated in VersaTREK® Culture System II that combines a liquid culture medium (para-JEM Broth), two growth supplements (para-JEM GS and para-JEM EYS), and, for contaminated samples, an antibiotic supplement (para-JEM AS) with a detection system that automatically incubates and monitor inoculated culture bottles. The sponges in para-JEM bottles provide support for growth and increase the surface area exposed to headspace oxygen.

2.5. DNA extraction

Briefly, the culture medium of the 33 positive bottles at VersaTREK® Culture System II was recovered and centrifuged at 5000 rpm for 20 min. The pellet was used to extract DNA as previously described (Bull et al., 2003).

2.6. MAP IS900 amplification

Presence of MAP genomic DNA was confirmed using PCR amplification of IS900 sequences as previously described (Bull et al., 2003).

2.7. Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD), and categorical variables as numbers and percentages. PD patients and HCs were compared by Student's t-test. Correlations among continuous variables were calculated as well. Optimal cut-off values were determined by setting the specificity at 95% and the corresponding sensitivity was calculated using ROC analysis. A p value equal or less than 0.05 was considered significant.

Statistic studies were carried out using GraphPad Prism 6.0 software (San Diego, CA, USA).

3. Results

3.1. ELISA

Humoral response against the selected peptides was evaluated both in PD patients and in HC. The highly conserved peptides were identified after Blast comparison analysis between ZnT human proteins and MAP 3865c proteins (Table 1). Regarding MAP3865c$_{207–219}$, a statistically significant difference was observed between the antibody positivity of PD patients and HC with a p value of 0.024 analyzed through T test (Fig. 1 A), however no significant difference in the humoral response was observed regarding the human homolog ZnT3$_{286–297}$ showing a p value of 0.70 (Fig. 1 B). Patients seropositivity to MAP3865c$_{207–219}$ was found in 17.5% of PD patients whereas only 2.5% of HC reacted against the selected peptide. Seropositivity against ZnT3$_{286–297}$ was found in 40% of PD patients and 40% of HC. Evaluation of antibody reactivity against MAP3865c$_{81–95}$ did not show statistical difference with a p-value of 0.13 (Fig. 1 C). No difference was observed as well between PD and HC when the human homolog ZnT3$_{135–149}$ was investigated with a p-value of 0.45 (Fig. 1 D). Seropositivity against MAP3865c$_{81–95}$ was seen in 52.5% of patients and 27.5% of HC. The human homologous ZnT3$_{135–149}$ revealed 42.5% of positivity in PD patients and 27.5% in HC. Regarding MAP3865c$_{44–59}$, no statistical difference was observed between PD patients and HC with a p value of 0.84 analyzed by the T test, showing a seropositivity of 42.5% in PD patients and 30% in HC (Fig. 2 A). Similarly, no significant difference in the humoral response against the human homolog ZnT10$_{34–49}$ in patients and controls was reported with a p-value of 0.64 (Fig. 2 B). Thirty per cent of PD patients reacted against ZnT10$_{34–49}$, a similar result, 32.5%, was seen in HC. Regarding MAP3865c$_{82–97}$, a statistically significant difference was observed between PD patients and HC with a p value of 0.0139 analyzed by T test (Fig. 2 C); On the other hand, no difference in the humoral response was observed for the human homolog ZnT10$_{72–87}$ showing a p value of 0.24 obtained by T test (Fig. 2 D). Seropositivity registered for MAP3865c$_{82–97}$ reached 47.5% in patients and 22.5% in HC., The seropositivity observed for ZnT10$_{72–87}$ was 37.5% in PD patients and 30% in HC. Finally, MAP3865c$_{230–244}$ revealed no statistical difference between PD patients and HC with a p value of 0.081 analyzed by T test (Fig. 3 A). Likewise, no significant difference in the humoral response was observed for the human homolog ZnT10$_{324–338}$ showing a p value of 0.16 (Fig. 3 B). The seropositivity observed for MAP3865c$_{230–244}$ was 40% in PD patients and 30% in HCs whereas the seropositivity observed for ZnT10$_{324–338}$ was 50% in PD patients and 30% in HCs (Table 2).

3.2. VersaTREK and PCR IS900 analysis

Forty PBMC samples derived from PD patients were incubated in the VersaTREK system, 33 of them resulted positive and 7 negative. The

Table 1
characteristics of selected peptides.

Peptide name	Sequence	Protein name	Organism
MAP3865c$_{207–219}$	RDALRILSESSP	MAP3865c	*Mycobacterium avium* subsp. *paratuberculosis*
ZnT3$_{286–297}$	RDVLRILMEGTP	ZnT3	*Homo sapiens*
MAP3865c$_{81–95}$	TYGWHRAEVFTAVA	MAP3865c	*Mycobacterium avium* subsp. *paratuberculosis*
ZnT3$_{135–149}$	TFGWHRSETLGALA	ZnT3	*Homo sapiens*
MAP3865c$_{44–59}$	NSIALLADAGHMLTDV	MAP3865c	*Mycobacterium avium* subsp. *paratuberculosis*
ZnT10$_{34–49}$	NSIALLSDSFNMLSDL	ZnT10	*Homo sapiens*
MAP3865c$_{82–97}$	TYGWHRAEVFTAVANA	MAP3865c	*Mycobacterium avium* subsp. *paratuberculosis*
ZnT10$_{72–87}$	TYGYARAEVVGALSNA	ZnT10	*Homo sapiens*
MAP3865c$_{230–244}$	AVDGVTGVHDLHVW	MAP3865c	*Mycobacterium avium* subsp. *paratuberculosis*
ZnT10$_{324–338}$	AVPGISSVHEVHIW	ZnT10	*Homo sapiens*