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Low levels of alpha-synuclein in peripheral tissues are related to clinical relapse in relapsing-remitting multiple sclerosis: a pilot cross-sectional study

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ABSTRACT

The protein alpha-synuclein (α-Syn) has been linked to neuroinflammatory conditions. We investigated whether the presence of α-Syn in peripheral tissues is a surrogate of brain inflammatory status in a small group of relapsing-remitting multiple sclerosis (RRMS) patients in a pilot cross-sectional study. Skin biopsies and peripheral blood were sampled from 34 healthy controls and 23 MS patients for measurement of α-Syn levels. Within the RRMS group 15 patients were in remission, and 8 patients were in the relapsing phase. The protein α-Syn was evaluated by means of immunohistochemistry and flow cytometry in skin and nucleated blood cells, respectively. In the skin, α-Syn levels were lower in relapsing MS than in the other groups, both in positive area (p = .021) and staining intensity (p = .004). In blood, the percentage of α-Syn-positive lymphocytes and monocytes were not statistically different between study groups. Moreover, the use of systemic steroids did not affect α-Syn positivity in MS-relapse patients. Finally, epidermic Langerhans cells did not stain positively for α-Syn. Overall, the levels of α-Syn positivity were lower in inflammatory relapse of RRMS patients when measured in peripheral tissues. We discuss the role of α-Syn levels in inflammation according to the obtained results.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) characterized by focal areas of neurological damage. The clinical course of MS includes motor, visual, and affective symptoms with varying degrees of disability [1]. Among the four clinical variants described, relapsing-remitting multiple sclerosis (RRMS) accounts for 80%–85% of total MS cases [2]. In this variant, inflammatory brain lesions accompanied by clinical symptoms (relapse) alternating with disease-free intervals (remission). Although the etiology of MS is still unknown, disease progression relies on a repeated inflammatory response to myelin antigens. Environmental factors, infectious agents, and genetics have been implicated as risk factors for developing the disease [1]. Cognitive and physical impairments are common features of chronic MS [3,4].

Diagnosis of relapsing MS is often complicated by the high variability in clinical presentation, the availability of auxiliary methods (such as MRI and oligoclonal bands), and the inconclusive results obtained by these methods in some patients. Nevertheless, clinical presentation relies on active brain inflammation, and a peripheral marker for neuroinflammation would be a useful tool for assessing relapse in MS. Among the putative markers, the neuronal protein alpha-synuclein (α-Syn) has been previously associated with inflammation and development of neurodegenerative diseases (NDD) [5–8]. The ubiquitous localization of α-Syn in peripheral tissues is advantageous because it avoids the ethical concerns of directly studying brain tissue in CNS disorders. Indeed, the presence α-Syn in peripheral nerve terminals [9], epidermis [6,10,11], and blood cells [12] has been explored in Parkinson's and other NDD. In multiple sclerosis, α-Syn has been studied directly in CNS lesions and cerebrospinal fluid (CSF). For example, studying postmortem brain samples from MS patients, Lu and collaborators found that the presence of α-Syn is restricted to active

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inflammatory lesions, but it is absent in chronic non-inflammatory tissue [13]. Also, different α-Syn levels in CSF have been reported between MS patients and control subjects [14,15], but until now, studies approaching variations in peripheral α-Syn related to inflammation in MS are lacking.

Before studying α-Syn as an inflammatory marker, this work proposes to address the true association of α-Syn with inflammatory settings. This is because, beyond the association of increased α-Syn levels and inflammation, some studies have shown that decreased α-Syn levels were also related to inflammation and dysfunctional immune responses [16–18]. For example, Ettle and collaborators found that deleting α-Syn before induction of experimental allergic encephalitis (EAE) in mice increased the numbers of infiltrating Th1 inflammatory cells and decreased the number of Treg anti-inflammatory cells in the CNS [19]. When α-Syn was silenced in the rat CNS by a slightly different approach, neuroinflammation and microglia activation through increased MHC-I expression caused specific dopaminergic cell degeneration [20]. In another manner, increased α-Syn expression in mouse macrophages and microglia caused impairments in autophagy and subsequent dysfunction in phagocytosis and cytokine release [21]. These results indicate that the functions of α-Syn and its changes associated with pathology are only partially understood. Beyond its known participation as a chaperone in neurotransmitter release, inflammatory implications have also been suggested in peripheral tissues. Specifically, α-Syn can shape the function of the immune system, participating in T lymphocyte development [17,18], modulating lymphocyte function [12], and regulating proper antigen processing in phagocytes and lymphocytes through autophagy [22].

Therefore, given the heterogeneity of the clinical presentation of MS, the neuroinflammatory basis of its symptoms and the dubious inflammatory properties associated with α-Syn in non-NDD, we designed this preliminary study to assert the association between α-Syn levels and inflammation in a small group of relapsing-MS patients and to ascertain the presence of α-Syn in peripheral tissues. The preliminary conclusions of this work will be the basis for further studies including a greater number of participants. Our initial hypotheses were: a) the α-Syn levels in skin and nucleated blood cells will be increased in symptomatic patients (MS-relapse) when compared to asymptomatic patients (MS-remission) and healthy controls; b) steroid treatment in MS-relapse will decrease α-Syn positivity in the studied tissues; c) cutaneous antigen-presenting cells (Langerhans cells or LC), as components of the immune system, will be positive for α-Syn.

2. Materials and methods

2.1. Participants

Skin biopsy and peripheral blood samples were taken from 34 healthy controls and 23 RRMS patients for MS diagnosis performed by an experimented neurologist according to the revised McDonald criteria. The RRMS group included 15 MS-remission and 8 MS-relapse patients (Table 1). Skin samples of 4-mm diameter were taken by punch biopsies from retroauricular skin. For MS-relapse patients, sampling was performed before and after the administration of intravenous methylprednisolone in a cumulative dose of 1000–1500 mg. Participants in the study were recruited from the Neurology departments of Central Hospital in San Luis Potosí and Western National Medical Center in Jalisco, México. All participants signed the informed consent form prior to the inclusion of their skin and blood samples in this study. The Ethics and Research Committee of the Central Hospital in San Luis Potosí, México, approved the study and consent forms. This study was performed under the code of ethics of the World Medical Association for experiments involving humans.

Table 1
Demographic characteristics of participants.

	Control	MS-Rem	MS-Rel	MS-Rel + IS	p-Value*
N =	34	15	8	8	–
M:F	13:21	10:5	4:4	2:6	–
Age in years (mean ± SD)	30.6 (± 7.7)	35.1 (± 8.4)	35.1 (± 8.1)	33.8 (± 7.8)	0.2120
Age in years (min-max)	20–49	23–50	22–44	22–44	–
EDSS score (mean ± SD)	–	3.6 (1.9)	2.2 (1.5)	2.3 (1.8)	0.1341
Steroids (%)	–	2 (13%)	–	8 (100%)	–

* F = 1.545 (age); F = 2.161 (EDSS); one-way analysis of variance.

2.2. Flow cytometry

Complete blood was diluted 1:3 in 0.01 M phosphate buffered saline (PBS) and then transferred to 15-mL tubes containing 3 mL of cold Histopaque at the bottom. The sample was centrifuged at 2500 RPM for 20 min at 10 °C. After this step, peripheral blood mononuclear cells (PBMC) were separated and washed with cold PBS and RBC lysis buffer. Following cell counting, 2×10^5 PBMC were fixed with 500 μL of Fix/Perm Solution (BD Biosciences) for 60 min at 4 °C. Following fixation, PBMC were incubated with mouse monoclonal anti-α-Syn antibody (BD Biosciences, 2 μL from a 1:5 dilution) for 60 min at 4 °C. In the next step, an FITC-coupled anti-mouse antibody (BD Biosciences, 2 μL from a 1:10 dilution) was added and incubated for 30 min, protected from light at 4 °C. Samples were analyzed in a FACS CANTO II cytometer (BD Biosciences, Franklin Lakes, NJ), and the percentage of α-Syn-positive cells were used for comparison purposes (Fig. 1A).

2.3. Immunohistochemistry and confocal microscopy

Skin biopsies were fixed in 4% formaldehyde, dehydrated, and embedded in paraffin blocks. For immunohistochemistry, 5-μm tissue slices were collected on electro-charged slides (Biocare Medical, Pacheco, CA). Dewaxing and rehydration were performed by xylene and ethanol rinses, respectively. Epitope recovery was performed in DIVA decloacker solution (Biocare Medical) in a pressure cooker at high power for 3 min. To avoid nonspecific staining, the following blockers were utilized: 3% H2O2 for endogenous peroxidase, Background Sniper (Biocare Medical) for nonspecific background staining, and the Avidin/Biotin Blocking Kit (Vector Laboratories Inc., Burlingame, CA) for endogenous biotin and biotin-binding proteins. The blockers were alternated with Tris-buffered saline + Tween-20 (TBST) rinses. Mouse monoclonal anti-α-Syn antibody (BD Biosciences) was used in a 1:100 dilution in TBST and incubated for 60 min, followed by 30 min with the streptavidin–biotin detection system (Dako, Agilent Technologies, Santa Clara, CA). A negative control without the primary antibody was included with each sample (Supp. Fig. 1). Peroxidase activity was visualized by incubating the sections with amino-ethylcarbazole solution for 9 min to obtain a positive red coloration. Samples were counterstained with Harris hematoxylin. Photomicrographs were taken on a light microscope equipped with a digital camera (Olympus, AmScope). Six fields per sample were captured at 40× magnification and digitally analyzed with Image Pro Plus 7 software. Two parameters were obtained from digital analysis of immunohistochemistry images: the α-Syn- positive area in pixels and the integrated optical density (IOD) in arbitrary units. The parameter IOD quantifies the intensity of positive spots, estimating the amount of marked α-Syn.

For confocal microscopy, samples were processed as described for immunohistochemistry, except for the use of peroxidase and biotin blockers. Following antigen recovery with DIVA decloacker solution and Background Sniper, 5-μm skin slices were incubated overnight at 4 °C with mouse anti-α-Syn (BD Biosciences, dilution 1:100) and rabbit

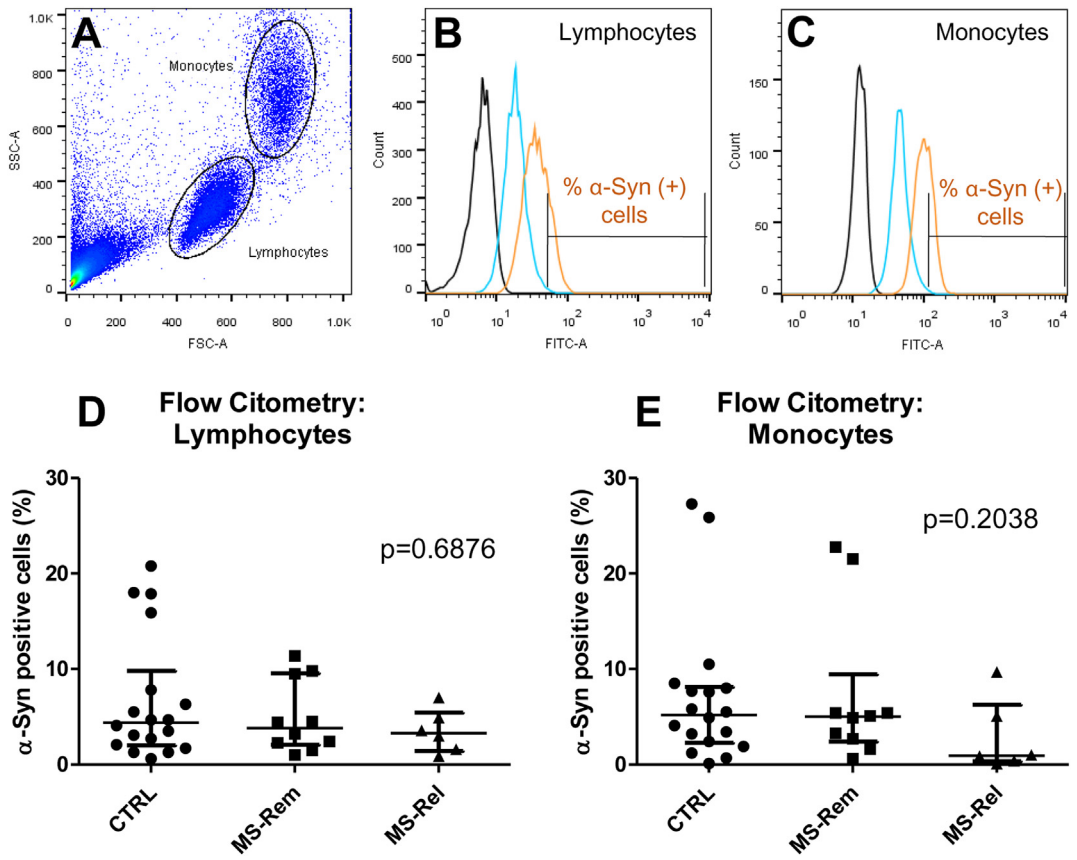

Fig. 1. α-Syn immunopositivity in PBMC. Staining for α-Syn was performed in PBMC (A); no differences were found in the percentage of positive cells in the lymphocyte (B and D) or monocyte (C and E) populations. Median ± IQR, Kruskal-Wallis test, each dot represents a single measure of a single subject. CTRL = healthy control, MS-Rem = multiple sclerosis remission phase, MS-Rel = multiple sclerosis relapse phase. B and C are representative histograms from a healthy control.

anti-CD1a (Abcam, Cambridge, MA, dilution 1:300) antibodies. In the next step, goat anti-mouse IgG coupled with Alexa Fluor 633 (dilution 1:100) and goat anti-rabbit IgG coupled with Alexa 488 (dilution 1:200) were incubated for 2 h at room temperature and light protected. Nuclei were stained with Sytox (Molecular Probes, Waltham, MA). The slides were covered with VectaShield mounting solution. Samples were analyzed in a confocal microscope (LEICA TCS SP2, Leica Microsystems GmbH, Wetzlar, Germany) for fluorescence at 40× magnification.

2.4. Statistical analysis

Statistical analysis was performed with GraphPad Prism 5 software. According to the normality test, our data showed a non-gaussian distribution. For comparisons between two groups, we performed a Mann–Whitney *U* test, and for multiple groups, a Kruskal–Wallis test with Dunn's post-test were employed instead. A *P*-value lower than 0.05 was considered for statistical significance.

3. Results

3.1. Patients

As mentioned, the RRMS group was divided in two subgroups, 15 MS-remission patients recruited in the clinical follow-up and 8 MS-relapse patients with clinical or radiologic signs of inflammatory activity. The description of participants is shown in Table 1. The scant number of patients included in this work was justified by the low-intermediate prevalence of MS in México and the exploratory design of the study [23]. The mean ages and EDSS scores were not significantly different between groups (EDSS scores [mean ± SD]: 3.6 ± 1.9 [MS-remission], 2.2 ± 1.5 [MS-relapse] and 2.3 ± 1.8 [MS-relapse + steroids]; F = 2.161, p = .1341, one-way ANOVA). The post-steroid samples were obtained after a cumulative dose of 1.5 g of intravenous methylprednisolone.

3.2. α-Syn in nucleated blood cells and epidermis

In blood tissue, the percentage of α-Syn-positive cells showed no significant difference between study groups in lymphocyte (p = .6876) and monocyte (p = .2038) samples, but a trend toward lower percentages in the MS-relapse group was observed (Fig. 1 and Suppl. Fig. 2).

In contrast, the epidermis showed lower α-Syn positivity in the MS-relapse group. The areas of α-Syn-positive cells were significantly lower in the MS-relapse group when compared with healthy controls (p = .0214) but statistically similar to the MS-remission group. The parameter IOD also showed the lowest values in the MS-relapse group when compared to the other study groups (p = .0041) (Fig. 2).

3.3. Effect of systemic steroids on α-syn positivity

Intravenous methylprednisolone is commonly used in patients with clinical signs of MS relapse. In this subgroup of patients, we evaluated the change in α-Syn positivity after acute treatment with steroids, but no change was found in any of the tissues studied when compared to pre-steroid samples (Fig. 3A–D).

3.4. α-Syn co-localization with langerhans cells

As a component of the immune system, LC numbers decreased after steroid treatment when compared to pre-steroid samples in the MS-

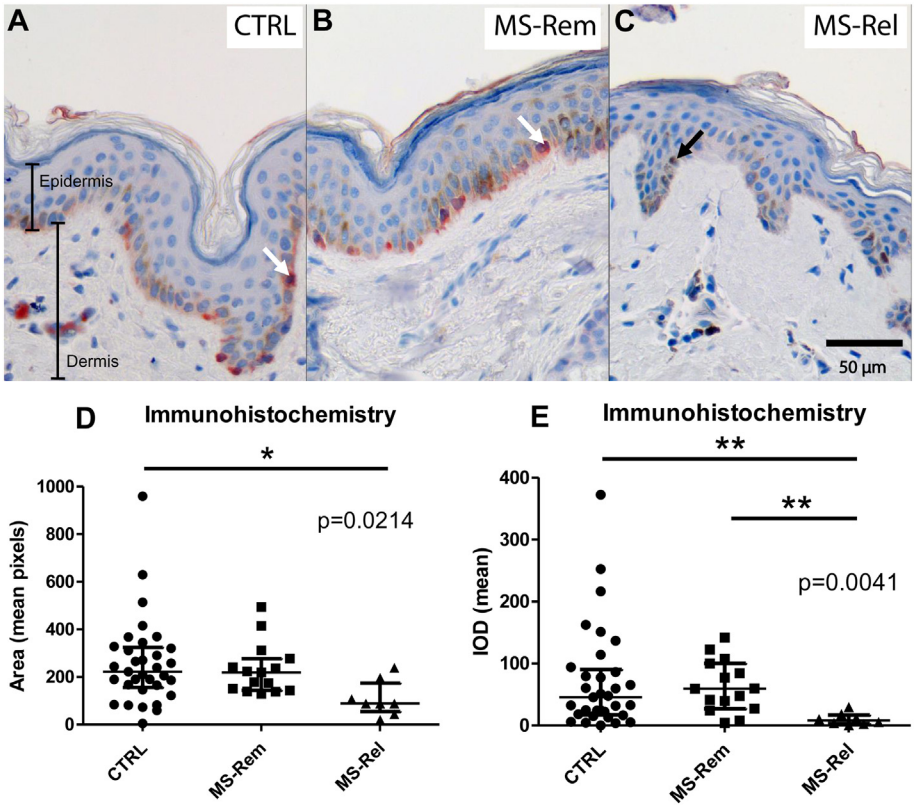

Fig. 2. α-Syn immunopositivity in the epidermis. Immunohistochemistry for α-Syn was performed in healthy controls (A), MS-remission patients (B) and MS-relapse patients (C). The marked area for α-Syn was lower in the MS-relapse group compared to healthy controls (D). The integrated optical density had lower values in MS-relapse patients when compared to MS-remission and healthy control values (E). Light microscopy, 40× magnification, white arrows = α-Syn positive cells, black arrows = melanin inclusions. Median ± interquartile range (IQR), Kruskal–Wallis test followed by Dunn's post-test. Each dot represents the mean value of six fields at 40× from a single participant. $*p < .05$, $**p < .01$. CTRL = healthy control, MS-Rem = multiple sclerosis remission phase, MS-Rel = multiple sclerosis relapsing phase.

relapse subgroup of patients (Fig. 3E–F). We found no spatial co-localization between LC and α-Syn markers. The α-Syn-positive cells were restricted to the basal layer, and LC were distributed along the whole epidermis (Fig. 3G–I).

4. Discussion

This preliminary approach explored the variations of peripheral α-Syn levels according to inflammatory status in MS patients. Our results showed that a) α-Syn levels in the epidermis had lower values in MS-relapse patients; b) treatment with steroids in MS-relapse did not change the rate of positivity to α-Syn; c) LC did not show positivity to α-Syn.

The main outcome of this study argues against considering α-Syn as an inflammation-related protein and shows the weak suitability of α-Syn as an inflammatory marker. First, we found lower positivity to α-Syn in the epidermis from MS-relapse patients when compared to MS-remission patients and healthy controls (Fig. 2). A similar but statistically nonsignificant trend was seen in nucleated blood cells (Fig.1). The pattern of staining of neuronal proteins in the skin (as a neural crest derived tissue) is quite specific. Indeed, we have studied other proteins in the skin and we found positive polygonal cells restricted mostly to basal epidermis in the case of α-Syn. By contrast, a cytoplasmic pattern involving the whole epidermis was observed for beta-tubulin, digitiform positive cells interspersed across epidermis for Langerin and nuclei-restricted positivity in external layers of epidermis for phosphorylated tau (Supp. Fig. 3). Also, it is important to mention that melanin expression is variable according to skin tone and it is easily identified as dark-brown irregular spots (Supp. Fig. 1). The red positive α-Syn marks are clearly differentiated from melanin with digital analysis of images. Within the flow cytometry approach, the possibility for contamination in sampled PBMC with α-synuclein from erythrocytes or RBC (also containing the protein) is avoided by means of the staining protocol itself, prior to analysis. In the protocol, the PBMC are separated from RBC by means of a density gradient followed by lysis of residual RBC with a hypotonic solution. For analysis, morphologic parameters (SSC and FSC) were utilized to gate a specific population clearly differentiated from cell fragments and small non-nucleated cells (Fig. 1A). Second, in the subgroup of MS-relapse patients, the use of steroids did not change the rate of positivity to α-Syn (Fig. 3, A-D), but the numbers of LC (as components of the immune system) were clearly decreased with steroids, suggesting that an effect of acute steroid treatment was indeed present (Fig. 3, E and F). Nevertheless, some drawbacks of this result must be mentioned. For example, MS-relapse was the smallest of the studied groups ($n = 8$) and, interestingly, those patients had the lowest measured α-Syn levels. Moreover, considering that we measured changes of protein positivity, the short time of intervention with steroids may have not been enough to see a change in α-Syn. Third, LC did not show positivity to α-Syn by confocal microscopy (Fig. 3, G-I). This finding is consistent with the above results. The α-Syn-positive cells were restricted to the basal layer of the epidermis, and we failed to identify them as LC by immunofluorescence, even with LC localized to this layer (Supp. Fig. 4). Moreover, Rodríguez-Leyva and collaborators previously identified a subgroup of α-synuclein positive cells as melanocytes, explaining their characteristic distribution in the basal layer of the epidermis [6].

In agreement with our findings, bibliographic evidence supports the existence of an inverse relationship between α-Syn levels and inflammatory conditions. For example, Antonelou and collaborators found that MS patients had lower α-Syn levels in CSF when compared to control patients with benign neurologic conditions such as migraine and sciatica [15]. Basic research in an animal model demonstrated that the deletion of α-Syn in mice before EAE induction increased the numbers of infiltrating CD4+ Th1 cells in the CNS and was accompanied by earlier appearance of clinical disease, suggesting that low or absent α-Syn levels were related to increased inflammation [19]. In another study, acute α-Syn silencing in mature nigral neurons resulted in increased microglia activation and recruitment of lymphocytes, leading to severe neurodegeneration [20]. Also, α-Syn deletion in lymphocytes increased the expression of activation markers CD69 and

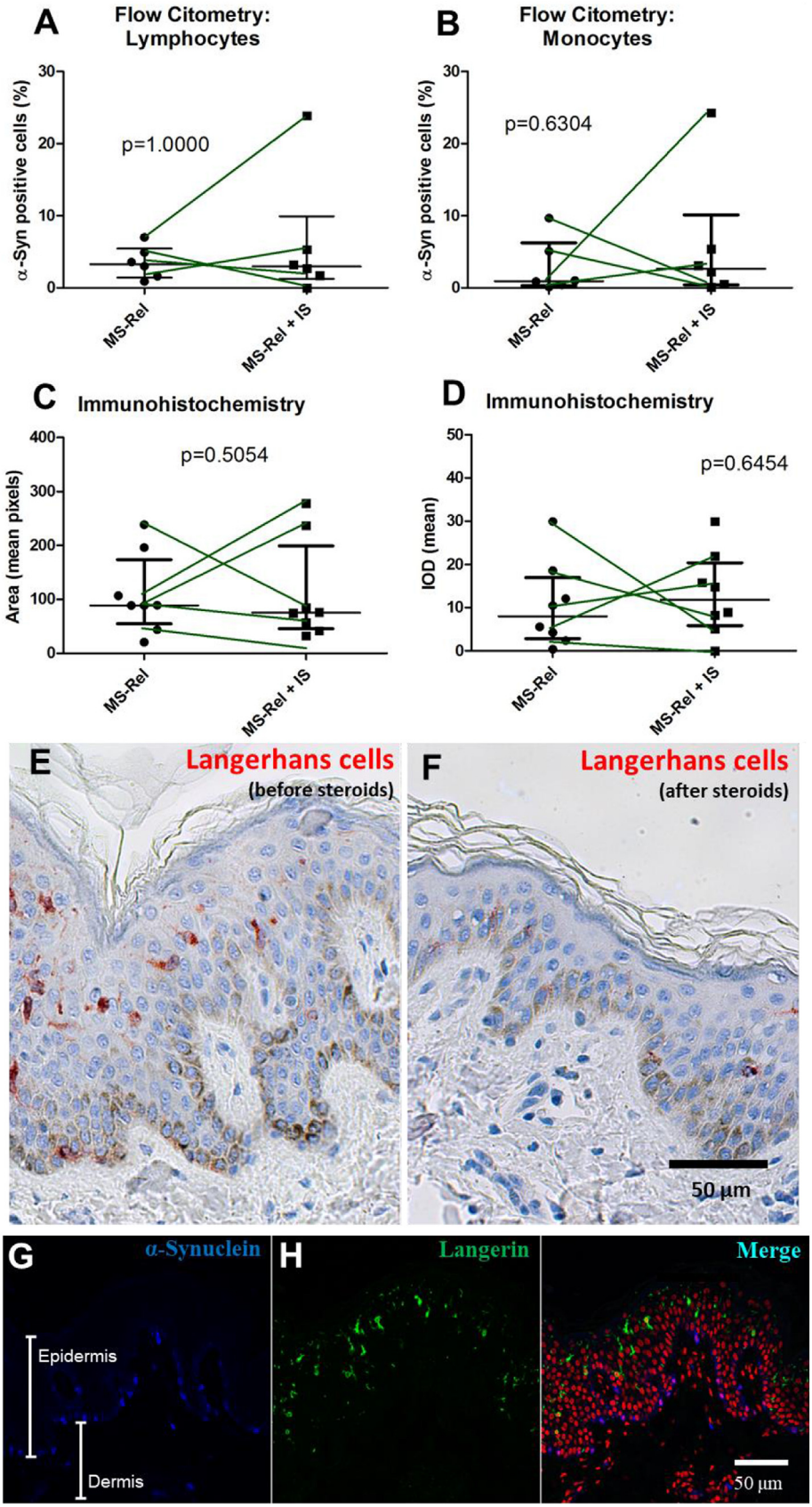

Fig. 3. α-Syn positivity and inflammation. Changes in α-Syn positivity were measured in MS-relapse patients before and after the administration of systemic steroids (connecting straight lines show the measures performed in the same patient). No difference was found in the percentage of α-Syn-positive cells between groups in lymphocytes (A) and monocytes (B). No difference was found in immunopositivity to α-Syn in the epidermis (C and D). Immunohistochemistry for LC in skin were performed before (E) and after (F) systemic steroid treatment in a single MS-relapse patient; positive cells stained red. Co-localization of α-Syn (blue, G) and Langerhans cells (green, H) was performed in skin from a healthy donor, but no co-localization was found (merge, I). Median ± IQR, Mann–Whitney *U* test (A-D). MS-Rel = multiple sclerosis relapse phase, IS = immunosuppresor (intravenous steroids). Light microscopy (E-F), confocal microscopy (G-I), 40× fields, nuclei stained with Sytox (red color in I). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

CD49d and decreased the number of anti-inflammatory T regulatory cells [18]. In the CNS, when α-Syn was depleted in microglia, these cells became reactive, with increased release of proinflammatory cytokines (TNF-α and IL-6) but dysfunctional in terms of an effective immune response [16]. Complimentary evidence suggested that increased α-Syn levels could even be a protective factor in clinical

scenarios where inflammation has deleterious effects [24,25]. Lastly, it is important to mention that the dysfunctional inflammatory phenotype associated with low α-Syn levels may become evident only when an inflammatory stimulus challenges immune homeostasis [26].

Given the results, the decreased α-Syn levels seen in inflammatory conditions seem to be in conflict with the increased α-Syn levels commonly associated with inflammation in neurodegenerative diseases. To address this discrepancy, we reviewed the participation of α-Syn in two divergent scenarios. First, the normal function of α-Syn is only partially understood. α-Syn works as a chaperone in intracellular vesicle dynamics, a critical step for neurotransmitter and cytokine release. Proper functioning of α-Syn is then related to neurotransmission, autophagy, inflammation, and phagocytosis [21,27–31]. Second, published evidence suggests the existence of two α-Syn functional variants based on its structural conformation. One is the regular α-Syn, mentioned above as a soluble amphipathic protein with many physiologic functions and a constant exchange rate [32–34]. The other is considered pathologic, an insoluble protein with a spectrum of modifications that make it prone to forming toxic aggregates, triggering inflammation and cell damage, and being implicated in the genesis of neurodegenerative diseases [28,29,35]. Then, pathologic variations in α-Syn structure corresponded to varying grades of inflammatory response measured through phagocytosis and microglia activation [36].

Our results suggest that the variant we measured is regular α-Syn. This variant appears to modulate inflammation, which would explain the lower α-Syn levels seen in inflammatory MS-relapse. Finally, we must state that our findings come from an initial study involving a limited number of patients and we expect to confirm our preliminary conclusions in a larger population.

5. Conclusion

In the present study we found lower α-Syn levels in skin from relapsing MS patients, suggesting that decreased modulatory properties of regular α-Syn contribute to the increased neuroinflammation seen in relapsing MS patients.

Funding/conflicts of interest

The authors declare that there are no conflicts of interest. MM received a scholarship from Consejo Nacional de Ciencia y Tecnología, México CVU 372299.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2018.11.003.

References

[1] C.A. Dendrou, L. Fugger, M.A. Friese, Immunopathology of multiple sclerosis, Nat. Rev. Immunol. 15 (2015) 545–558, https://doi.org/10.1038/nri3871.
[2] C. Schmidt, Biology: a degenerative affliction, Nature 540 (2016) S2–S3, https://doi.org/10.1038/540S2a.
[3] B. Cullen, D.J. Smith, I.J. Deary, J.J. Evans, J.P. Pell, The “cognitive footprint” of psychiatric and neurological conditions: cross-sectional study in the UK Biobank cohort, Acta Psychiatr. Scand. 135 (2017) 593–605, https://doi.org/10.1111/acps.12733.
[4] F. Cáceres, S. Vanotti, S. Rao, RECONEM Workgroup, Epidemiological characteristics of cognitive impairment of multiple sclerosis patients in a Latin American country, J. Clin. Exp. Neuropsychol. 33 (2011) 1094–1098, https://doi.org/10.1080/13803395.2011.603690.
[5] N. Malek, D. Swallow, K.A. Grosset, O. Anichtchik, M. Spillantini, D.G. Grosset, Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease - a systematic review, Acta Neurol. Scand. 130 (2014) 59–72, https://doi.org/10.1111/ane.12247.
[6] I. Rodriguez-Leyva, E. Chi-Ahumada, M. Mejía, J.P. Castanedo-Cazares, W. Eng, S.K. Saikaly, J. Carrizales, T.D. Levine, R.A. Norman, M.E. Jimenez-Capdeville, The presence of alpha-synuclein in skin from melanoma and patients with Parkinson's disease, Mov. Disord. Clin. Pract. 4 (2017) 724–732, https://doi.org/10.1002/mdc3.12494.
[7] G. Codolo, N. Plotegher, T. Pozzobon, M. Brucale, I. Tessari, L. Bubacco, M. de Bernard, Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies, PLoS One 8 (2013) e55375, , https://doi.org/10.1371/journal.pone.0055375.
[8] H.-J. Lee, J.-E. Suk, C. Patrick, E.-J. Bae, J.-H. Cho, S. Rho, D. Hwang, E. Masliah, S.-J. Lee, Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies, J. Biol. Chem. 285 (2010) 9262–9272, https://doi.org/10.1074/jbc.M109.081125.
[9] V. Donadio, A. Incensi, G. Rizzo, C. Scaglione, S. Capellari, E. Fileccia, P. Avoni, R. Liguori, Spine topographical distribution of skin α-Synuclein deposits in idiopathic Parkinson disease, J. Neuropathol. Exp. Neurol. 76 (2017) 384–389, https://doi.org/10.1093/jnen/nlx021.
[10] I. Rodríguez-Leyva, E.G. Chi-Ahumada, J. Carrizales, M. Rodríguez-Violante, S. Velázquez-Osuna, V. Medina-Mier, M.G. Martel-Gallegos, S. Zarazúa, L. Enríquez-Macías, A. Castro, A.L. Calderón-Garcidueñas, M.E. Jiménez-Capdeville, Parkinson disease and progressive supranuclear palsy: protein expression in skin, Ann. Clin. Transl. Neurol. 3 (2016) 191–199, https://doi.org/10.1002/acn3.285.
[11] I. Rodríguez-Leyva, A.L. Calderón-Garcidueñas, M.E. Jiménez-Capdeville, A.A. Rentería-Palomo, H.G. Hernandez-Rodriguez, R. Valdés-Rodríguez, C. Fuentes-Ahumada, B. Torres-Álvarez, J. Sepúlveda-Saavedra, A. Soto-Domínguez, M.E. Santoyo, J.I. Rodriguez-Moreno, J.P. Castanedo-Cázares, α-Synuclein inclusions in the skin of Parkinson's disease and parkinsonism, Ann. Clin. Transl. Neurol. 1 (2014) 471–478, https://doi.org/10.1002/acn3.78.
[12] T. Colasanti, M. Vomero, C. Alessandri, C. Barbati, A. Maselli, C. Camperio, F. Conti, A. Tinari, C. Carlo-Stella, L. Tuosto, D. Benincasa, G. Valesini, W. Malorni, M. Pierdominici, E. Ortona, Role of alpha-synuclein in autophagy modulation of primary human T lymphocytes, Cell Death Dis. 5 (2014) e1265, https://doi.org/10.1038/cddis.2014.211.
[13] J.-Q. Lu, Y. Fan, A.P. Mitha, R. Bell, L. Metz, G.R.W. Moore, V.W. Yong, Association of alpha-synuclein immunoreactivity with inflammatory activity in multiple sclerosis lesions, J. Neuropathol. Exp. Neurol. 68 (2009) 179–189, https://doi.org/10.1097/NEN.0b013e318196e905.
[14] H. Wang, K. Wang, W. Xu, C. Wang, W. Qiu, X. Zhong, Y. Dai, A. Wu, X. Hu, Cerebrospinal fluid α-synuclein levels are elevated in multiple sclerosis and neuromyelitis optica patients during replase, J. Neurochem. 122 (2012) 19–23, https://doi.org/10.1111/j.1471-4159.2012.07749.x.
[15] R.C. Antonelou, E. Emmanouilidou, G. Gasparinatos, T. Velona, K.I. Voumvourakis, L. Stefanis, Decreased levels of alpha-synuclein in cerebrospinal fluid of patients with clinically isolated syndrome and multiple sclerosis, J. Neurochem. 134 (2015) 748–755, https://doi.org/10.1111/jnc.13163.
[16] S.A. Austin, A.M. Floden, E.J. Murphy, C.K. Combs, α-Synuclein expression modulates microglial activation phenotype, J. Neurosci. 26 (2006) 10558–10563, https://doi.org/10.1523/JNEUROSCI.1799-06.2006.
[17] W. Xiao, A. Shameli, C.V. Harding, H.J. Meyerson, R.W. Maitta, Late stages of hematopoiesis and B cell lymphopoiesis are regulated by α-synuclein, a key player in Parkinson's disease, Immunobiology 219 (2014) 836–844, https://doi.org/10.1016/j.imbio.2014.07.014.
[18] A. Shameli, W. Xiao, Y. Zheng, S. Shyu, J. Sumodi, H.J. Meyerson, C.V. Harding, R.W. Maitta, A critical role for alpha-synuclein in development and function of T lymphocytes, Immunobiology 221 (2015) 333–340, https://doi.org/10.1016/j.imbio.2015.10.002.
[19] B. Ettle, K. Kuhbandner, S. Jörg, A. Hoffmann, J. Winkler, R.A. Linker, α-Synuclein deficiency promotes neuroinflammation by increasing Th1 cell-mediated immune responses, J. Neuroinflammation 13 (2016) 201, , https://doi.org/10.1186/s12974-016-0694-4.
[20] M.J. Benskey, R.C. Sellnow, I.M. Sandoval, C.E. Sortwell, J.W. Lipton, F.P. Manfredsson, Silencing alpha synuclein in mature nigral neurons results in rapid neuroinflammation and subsequent toxicity, Front. Mol. Neurosci. 11 (2018) 1–21, https://doi.org/10.3389/fnmol.2018.00036.
[21] S.J. Gardai, W. Mao, B. Schüle, M. Babcock, S. Schoebel, C. Lorenzana, J. Alexander, S. Kim, H. Glick, K. Hilton, J.K. Fitzgerald, M. Buttini, S.S. Chiou, L. McConlogue, J.P. Anderson, D.B. Schenk, F. Bard, J.W. Langston, T. Yednock, J.A. Johnston, Elevated alpha-synuclein impairs innate immune cell function and provides a potential peripheral biomarker for Parkinson's disease, PLoS One 8 (2013) 1–21, https://doi.org/10.1371/journal.pone.0071634.
[22] A.S. Harms, S. Cao, A.L. Rowse, A.D. Thome, X. Li, L.R. Mangieri, R.Q. Cron, J.J. Shacka, C. Raman, D.G. Standaert, MHCII is required for α-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration, J. Neurosci. 33 (2013) 9592–9600, https://doi.org/10.1523/JNEUROSCI.5610-12.2013.
[23] V.M. Rivera, M.T. Medina, R.M. Duron, M.A. Macias, Multiple sclerosis care in Latin America, Neurology 82 (2014) 1660–1661, https://doi.org/10.1212/WNL.0000000000000376.
[24] V. Labrie, P. Brundin, Alpha-synuclein to the rescue: Immune cell recruitment by alpha-synuclein during gastrointestinal infection, J. Innate Immun. 9 (2017) 437–440, https://doi.org/10.1159/000479653.

[25] E.L. Beatman, A. Massey, K.D. Shives, K.S. Burrack, M. Chamanian, T.E. Morrison, J.D. Beckham, Alpha-synuclein expression restricts RNA viral infections in the brain, J. Virol. 90 (2015) 2767–2782, https://doi.org/10.1128/JVI.02949-15.
[26] M.Y. Golovko, E.J. Murphy, Brain prostaglandin formation is increased by alpha-synuclein gene-ablation during global ischemia, Neurosci. Lett. 432 (2008) 243–247, https://doi.org/10.1016/j.neulet.2007.12.031.
[27] J.T. Bendor, T.P. Logan, R.H. Edwards, The function of α-synuclein, Neuron 79 (2013) 1044–1066, https://doi.org/10.1016/j.neuron.2013.09.004.
[28] J. Burré, M. Sharma, T. Tsetsenis, V. Buchman, M.R. Etherton, T.C. Südhof, Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro, Science 329 (2010) 1663–1667, https://doi.org/10.1126/science.1195227.
[29] J. Goers, A.B. Manning-Bog, A.L. McCormack, I.S. Millett, S. Doniach, D.A. Di Monte, V.N. Uversky, A.L. Fink, Nuclear localization of alpha-synuclein and its interaction with histones, Biochemistry 42 (2003) 8465–8471, https://doi.org/10.1021/bi0341152.
[30] A. Villar-Piqué, T. Lopes Da Fonseca, T.F. Outeiro, Structure, function and toxicity of alpha-synuclein: the Bermuda triangle in synucleinopathies, J. Neurochem. 139 (Suppl) (2016) 240–255, https://doi.org/10.1111/jnc.13249.
[31] A. Abeliovich, Y. Schmitz, I. Fariñas, D. Choi-Lundberg, W.H. Ho, P.E. Castillo, N. Shinsky, J.M. Verdugo, M. Armanini, A. Ryan, M. Hynes, H. Phillips, D. Sulzer, A. Rosenthal, Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system, Neuron 25 (2000) 239–252 http://www.ncbi.nlm.nih.gov/pubmed/10707987.
[32] T. Bartels, J.G. Choi, D.J. Selkoe, α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation, Nature 477 (2011) 107–110, https://doi.org/10.1038/nature10324.
[33] L. Fonseca-Ornelas, C. Schmidt, A.R. Camacho-Zarco, C.O. Fernandez, S. Becker, M. Zweckstetter, Small molecule-induced soluble oligomers of α-synuclein with helical structure, Chemistry (2017), https://doi.org/10.1002/chem.201703001.
[34] M.H.R. Ludtmann, P.R. Angelova, N.N. Ninkina, S. Gandhi, V.L. Buchman, A.Y. Abramov, Monomeric alpha-synuclein exerts a physiological role on brain ATP synthase, J. Neurosci. 36 (2016) 10510–10521, https://doi.org/10.1523/JNEUROSCI.1659-16.2016.
[35] A. Gustot, J.I. Gallea, R. Sarroukh, M.S. Celej, J.-M. Ruysschaert, V. Raussens, Amyloid fibrils are the molecular trigger of inflammation in Parkinson's disease, Biochem. J. 471 (2015) 323–333, https://doi.org/10.1042/BJ20150617.
[36] A. Hoffmann, B. Ettle, A. Bruno, A. Kulinich, A.-C. Hoffmann, J. von Wittgenstein, J. Winkler, W. Xiang, J.C.M. Schlachetzki, Alpha-synuclein activates BV2 microglia dependent on its aggregation state, Biochem. Biophys. Res. Commun. 479 (2016) 881–886, https://doi.org/10.1016/j.bbrc.2016.09.109.