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Short Communication

Ultrastructural changes in peripheral blood leukocytes in α-synuclein knockout mice

Hammad Tashkandi, Afshin Shameli, Clifford V. Harding, Robert W. Maitta*

University Hospitals Cleveland Medical Center, Case Western Reserve University School of Medicine, Cleveland, OH, United States of America

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ABSTRACT

Effects of α-synuclein deficiency on cellular blood components have not been extensively investigated. This study evaluated ultrastructural changes of leukocytes in α-synuclein knockout (KO) mice using electron microscopy (EM). The following ultrastructural characteristics were quantified in leukocytes: mitochondria, primary granules, specific granules (SG), Golgi apparatus (GA), inclusions, rough-endoplasmic reticulum (RER), smooth-endoplasmic reticulum (SER), and cellular projections (CP). EM showed increased numbers or amounts of SG, inclusions, and SER in KO group (5.3 ± 4.5 in WT vs. 14.1 ± 10.3 in KO, $p = 0.02$; 0.4 ± 0.9 in WT vs. 3.2 ± 2.8 in KO, $p = 0.007$; and 7.7 ± 6.7 in WT vs. 17.7 ± 12.2 in KO, $p = 0.03$, respectively). Although CP number was not significantly different between the two groups (13.4 ± 5.3 in WT vs. 16.3 ± 7.5 in KO, $p = 0.32$), their size and shapes were altered in KO mice. Notably, findings occurred in the setting of significant lymphopenia. α-Synuclein deficiency leads to changes in size and shape of secretory particles and increases in SER, SG, and inclusions, indicating a potential role for α-synuclein in vesicular trafficking in leukocytes. Further studies are needed to elucidate functions mediated by α-synuclein.

1. Introduction

α-Synuclein is a small protein (14 kDa) that is important to normal brain function and in certain conditions undergoes aberrant protein conformational changes that lead to the pathologic findings seen in Lewy bodies characteristic of Parkinson's disease [1]. The function of this molecule in the central nervous system still remains to be fully elucidated; but it appears to play important roles in promoting the health of the presynaptic terminal, neurotransmitter release in response to stimuli, and neuronal plasticity [2]. It can also have inhibitory roles, as seen in dopaminergic neurotransmitter release [3,4]. This protein is co-localized at or near the plasma membrane and cytoplasmic membranous structures, and this localization may mediate its neuronal functional roles.

It has also become evident that this protein is also well expressed in the hematopoietic system, but its functions in this system remain obscure. Reports have indicated that α-synuclein expression is highest in megakaryocytes, platelets, red blood cells (RBC), and erythroid precursors [5,6]. α-Synuclein expression has also been reported to be a specific marker of neoplastic changes in the megakryocytic lineage [7]. Platelets have been shown to have the highest concentration of α-synuclein per milligram of cellular protein in peripheral blood [8], and time-dependent α-synuclein concentration increases in plasma supernatant of stored platelets may be indicative of changes that platelets undergo while in storage [9]. Importantly, α-synuclein in platelets has been shown to inhibit α-granule release in a calcium dependent manner [10]. In addition, α-synuclein deficiency leads to production of small circulating platelets and significant anemia [11].

In leukocytes, the function of α-synuclein appears to be critical since its deficiency leads to severe paucity in the number of mature B and T lymphocytes, lack of immunoglobulin class switching in response to stimuli (with a resulting absence of IgG), and abnormal development of T helper and T regulatory cells [11,12]. In light of these observations that α-synuclein leads to quantitative and functional impairment of lymphocytes, we proceeded to look at the ultrastructural effects of α-synuclein deficiency on leukocytes by electron microscopy analysis of peripheral blood samples from α-synuclein knockout (KO) mice.

2. Methods

2.1. Mice

α-Synuclein$^{-/-}$ mice B6;129X1-Snca^{tm1Ros1} (stock 003692) (KO), and age- and sex-matched (8–10 week old) *α-synuclein*$^{+/+}$ B6;129SF2/J

* Corresponding author at: Department of Pathology, University Hospitals Cleveland Medical Center, Case Western Reserve University School of Medicine, Andrews 647A, PTH 5077, 11100 Euclid Avenue, Cleveland, OH 44106, United States of America.
E-mail address: robert.maitta@case.edu (R.W. Maitta).

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wild type (WT) mice were obtained from Jackson Laboratory (Bar Harbor, ME) [11]. Mice were housed in pathogen-free conditions at room temperature with standard night/light cycle, standard diet and water ad libitum. Animal protocols were approved by the Case Western Reserve University Institutional Animal Care and Use Committee.

2.2. Blood sampling and CBC

Mouse blood samples (200 µL) were collected from the tail vein in heparinized capillary tubes (Fisher Scientific, Pittsburgh, PA) and used for peripheral blood smears, manual blood counts, EM, and automated complete blood counts (CBC). The latter was performed as previously described [11].

2.3. Transmission electron microscopy

Preparation of cells for electron microscopy was done as previously described [13]. Blood samples from five WT and five KO mice were fixed by immersion in quarter-strength Karnovsky fixative solution for 2 h at room temperature. Specimens were washed and post-fixed for 2 additional hours in an unbuffered 1:1 mixture of 2% osmium tetroxide and 3% potassium ferrocyanide. Specimens were subsequently rinsed with distilled water followed by immersion overnight in an acidified solution of 0.25% uranyl acetate. Specimens were washed a second time in distilled water, dehydrated in ascending concentrations of ethanol, passed through propylene oxide, and embedded in a Poly/Bed 812 embedding media (Polysciences, Warrington, PA). Thin sections were obtained using a RMC MT6000-XL ultramicrotome (Boeckeler Instruments, Inc., Tucson, AZ). Sections were mounted on Gilder square 300 mesh nickel grids (Electron Microscopy Sciences, Hatfield, PA) followed by staining with acidified methanolic uranyl acetate and stable lead staining solution. Sections were subsequently coated on a Denton DV-401 carbon coater (Denton Vacuum LLC, Moorestown, NJ), and analyzed in a JEOL 1200EX electron microscope (JEOL Ltd., Tokyo, Japan). The following morphological and ultrastructural characteristics were recorded from all leukocyte subsets [14–16]: cell diameter, cell perimeter, number of mitochondria, primary granules (PG), specific granules (SG), Golgi apparatus, inclusions, rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and cellular projections (CP). Separately, peripheral blood smears were obtained and used to derive manual 100-cell differential leukocyte count using light microscopy. Transmission electron microscopy and light microscopy analyses were performed by two pathologists independently.

2.4. Cell measurements

Photoshop CC version 19.1.1 (Adobe Systems, San Jose, CA) was used to obtain measurements. Cell diameter and perimeter were obtained by using the select tool that was customized to micrometer units using the scale bar in the EM images.

2.5. Statistical analysis

All statistics were performed using Prism 6 (GraphPad Software Inc., La Jolla, CA). Results are presented as mean ± SD. Intergroup data comparisons were performed using Mann Whitney test and unpaired *t*-test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Cell differential

Manual 100-cell differential count showed relative lymphopenia (88.4 ± 7.9% in WT vs. 69.2 ± 13.5% in KO, $p = 0.03$) and neutrophilia (5.0 ± 3.6% in WT vs. 21.2 ± 8.0% in KO, $p = 0.006$) in KO mice compared to WT controls (Table 1). These data correlated with automated CBC results and those previously published in this mouse strain [11]. KO mice had a marked absolute lymphopenia ($3.8 \pm 1.0 \times 10^9$/L in WT vs. $2.9 \pm 1.0 \times 10^9$/L in KO, $p = 0.029$) and a relative percent neutrophilia, the latter likely secondary to the reduced number of lymphocytes since the absolute neutrophil counts were similar between KO and WT mice. Furthermore, KO animals appeared to have a normocytic anemia phenotype since RBC, hemoglobin (Hgb) and hematocrit (Hct) were lower in KO mice compared to WT ($9.1 \pm 0.8 \times 10^{12}$/L vs. $10.8 \pm 0.8 \times 10^{12}$/L, $p < 0.0001$; 14.3 ± 0.7 g/dL vs. 16.1 ± 1.2 g/dL, $p = 0.0005$; and 47.8 ± 4.4% vs. 57.8 ± 4.9%, $p < 0.0001$ respectively) in the context of normal mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) (Table 1B). There were no statistical differences in monocyte counts between the two mouse groups while basophils and eosinophils could not be detected in the majority of animals from both groups (mean of 0 and SD 0, data not shown).

Table 1
Cell differentials of peripheral blood samples from KO mice and age-matched WT controls.

	WT	KO	*p* value
A. Manual differential using peripheral blood smears			
Lymphocytes %	88.4 ± 7.9	69.2 ± 13.5	**0.03**
Eosinophils %	0.6 ± 1.2	0.8 ± 0.75	0.8
Neutrophils %	5.0 ± 3.6	21.2 ± 8.0	**0.006**
Monocytes %	8.4 ± 3.8	8.8 ± 5.7	0.44
Basophils %	0	0	
Bands %	0	0	
Based on 100 cell counts of smears from 5 mice/group (Mean ± SD)			
B. Automated cell blood count			
White blood cells ($\times 10^9$/L)	4.7 ± 1.2	4.0 ± 1.4	0.210
Neutrophils (%)	14.7 ± 5.4	19.3 ± 5.0	**0.026**
Neutrophils ($\times 10^9$/L)	0.7 ± 0.3	0.8 ± 0.3	0.525
Lymphocytes (%)	80.0 ± 5.7	72.8 ± 4.1	**0.003**
Lymphocytes ($\times 10^9$/L)	3.8 ± 1.0	2.9 ± 1.0	**0.029**
Monocytes (%)	5.0 ± 1.2	7.3 ± 2.9	**0.030**
Monocytes ($\times 10^9$/L)	0.2 ± 0.1	0.3 ± 0.2	0.325
Erythrocytes ($\times 10^{12}$/L)	10.8 ± 0.8	9.1 ± 0.8	< **0.0001**
Hgb (g/dL)	16.1 ± 1.2	14.3 ± 0.7	**0.0005**
Hct (%)	57.8 ± 4.9	47.8 ± 4.4	< **0.0001**
MCV (fL)	53.4 ± 3.0	52.5 ± 1.5	0.354
MCH (pg)	15.0 ± 1.2	15.7 ± 1.6	0.210
MCHC (g/dL)	28.0 ± 1.9	30.2 ± 3.3	0.083
RDW (%)	17.6 ± 1.1	17.1 ± 0.6	0.234
Platelets ($\times 10^9$/L)	624.5 ± 59.3	660.9 ± 91.4	0.282
Mean platelet volume (fL)	4.1 ± 0.3	3.9 ± 0.1	**0.017**
Based on counts from 11 mice/group (Mean ± SD)			

3.2. Cell ultrastructure

Transmission electron microscopy comparison of the leukocytes of WT and KO mice indicated that the latter had marked increases in the number of SG, inclusions and SER compared to the former (5.3 ± 4.5 in WT vs. 14.1 ± 10.3 in KO, $p = 0.02$; 0.4 ± 0.9 in WT vs. 3.2 ± 2.8 in KO, $p = 0.007$; and 7.7 ± 6.7 in WT vs. 17.7 ± 12.2 in KO, $p = 0.03$, respectively) (Table 2). Analysis of the number of CPs between the two groups showed no significant difference between leukocytes of WT and KO mice (13.4 ± 5.3 in WT vs. 16.3 ± 7.5 in KO, $p = 0.32$). Of note, sizes and shapes of CPs were altered in KO mice (Fig. 1). No differences were noted in other organelles between the two groups (data not shown).

3.3. Cell dimensions

Cells from KO and WT mice were measured to determine changes in dimensions caused by α-synuclein deficiency. KO mice leukocytes had a > 14% larger diameter compared to WT leukocytes (6.8 ± 1.0 vs.