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Short Communication

Effects of lipoic acid on migration of human B cells and monocyte-enriched peripheral blood mononuclear cells in relapsing remitting multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a disease of the central nervous system characterized by inflammation and demyelination resulting in clinical disability. The rodent MS model suggests that infiltration of monocytes and B cells contributes to disease pathogenesis. Here, we compared the migratory capacity of human monocytes and B cells from healthy control (HC) and relapsing-remitting MS (RRMS) subjects, with or without lipoic acid (LA) treatment. Basal migration of monocyte-enriched PBMCs from RRMS subjects is significantly higher than HC PBMCs. LA treatment significantly inhibits monocyte and B cell migration in both cohorts, and may thus be therapeutically effective for treatment of MS.

1. Introduction

Multiple Sclerosis (MS) is characterized by the invasion of inflammatory cells across the blood brain barrier (BBB) leading to demyelination of the central nervous system (CNS) and axonal lesions (Breij et al., 2008). Though there are multiple clinical subtypes, most are initially diagnosed with relapsing subtype, RRMS. Disability from RRMS results from incomplete recovery after an exacerbating relapse. The majority will progress to a progressive phase, Secondary Progressive MS (SPMS). The pathophysiology of MS is complex, involving a variety of cell types including immune cells. In order to cause inflammation and damage, leukocytes must first migrate across the BBB into the CNS through cytokine and chemokine sensing (Agrawal and Yong, 2007). Several disease-modifying drugs (DMDs) target inflammatory responses to halt disease progression in RRMS patients. However, many DMDs have harsh side effects or are not effective, causing discontinuation rates upwards of 20% (Berger, 2011). Thus, there is a critical need for more treatments that are effective, tolerable and safe for the treatment of MS.

Lipoic acid (LA) is a well-tolerated dietary supplement and has been used for treatment of diabetic neuropathy in Germany for many years (Ziegler et al., 2004; Ziegler et al., 2016). It has been shown to reduce monocyte migration across the BBB into the CNS in rodent experimental autoimmune encephalitis (EAE) models (Marracci et al., 2002; Schreibelt et al., 2006). Migration of monocytes across the BBB causes the production of reactive oxygen species (ROS) which are thought to decrease the integrity of the BBB and induce further monocyte migration. LA has been shown to be an effective ROS scavenger, and is able to reduce or delay pathologies caused by ROS in diseases such as diabetic neuropathy and EAE (Packer et al., 2001). Additionally, in a recent clinical trial, LA has been seen to be therapeutically effective in reducing brain atrophy in MS patients (Spain et al., 2017). Both monocytes and B cells have been shown to additionally contribute to the development of RRMS by increasing local pro-inflammatory cytokines, such as IL-6, and inducing pathogenic T cell proliferation (Barr et al., 2012; Ireland et al., 2014; Fiedler et al., 2017). Specifically, B cells have been found to directly support a Th17 response to neuro-antigens (Ireland et al., 2016). The abnormal B cell cytokine profile may be mediating the pro-inflammatory responses of the disease-related T cells through "bystander activation" as suggested by a human study that saw a significant reduction in pro-inflammatory T cell responses when B cells were diminished (Bar-Or et al., 2010). This, combined with data shown herein, suggest that LA may be effective for treating MS, in part, by inhibiting or reducing monocyte and B cell migration into the CNS.

In this study, we compared the migratory capacity of human B cells and monocyte-enriched peripheral blood mononuclear cells (PBMCs) from healthy control (HC) and RRMS subjects. We further investigated the effects of LA on the migration of these cells and whether these effects differ in cells from RRMS subjects compared to HC.

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2. Materials and methods

2.1. Subjects

This study was approved by the joint VA Portland Health Care System (VAPORHCS) and Oregon Health & Science University (OHSU) institutional review boards (IRB #2993, IRB #8908) prior to initiation.

To isolate PBMCs, whole blood was diluted 1:1 (v/v) in PBS + 2% heat inactivated fetal bovine serum (HI FBS, Gibco/Thermo Fisher, Waltham, MA), centrifuged at 200 x g (Beckman Coulter, Brea, CA) for 10 min and plasma removed. Following Ficoll gradient separation in 50 mL SepMate tubes (Stemcell Technologies, Cambridge, MA), PBMCs were isolated following the manufacture's protocol. PBMCs were washed 2 × in EasySep buffer (Stemcell Technologies) and 1 × in wash buffer, RPMI + L-Glutamine (Gibco/Thermo Fisher). Cells were counted and checked for viability before being cryopreserved in liquid nitrogen (Salinthone et al., 2008).

2.2. Monocyte enrichment and migration assay

PBMCs were thawed in a 37 °C water bath, treated with 0.6 mg/mL DNase I (Roche, Indianapolis, IN) for 15 min at 37 °C and 5% CO_2, counted and centrifuged at 300 × *g* for 10 min. PBMCs were then re-suspended in monocyte enrichment media, RPMI + L-Glutamine, 1 × Pen/Strep, 5 mM glucose (Sigma-Aldrich, St Louis, MO), and plated in a 24 well plate (5×10^6 cells per well). The cells were incubated for 90 min at 37 °C and 5% CO_2. Non-adherent cells were removed by washing 3 × with wash buffer and visually inspected at 200 ×. These monocyte-enriched PBMCs were lifted from well bottom with cold PBS, counted and assessed for viability. 0.6 mL of monocyte enrichment media containing 20 ng/mL C−C motif chemokine 2 (CCL2) (eBioscience, San Diego, CA) was placed in the bottom chamber of 8 μm pore transwell plates (Corning International, Corning, NY) and left to equilibrate for 90 min at 37 °C/5% CO_2. Null control chambers received no CCL2. Monocyte-enriched PBMCs were re-suspended in the top chamber in 0.1 mL RPMI media at 5×10^5 monocytes/mL and pre-treated with 100 μg/mL of LA for 10 min. Control aliquots (Null, UNT and CCL2) were not pre-stimulated with LA. The transwell plates were incubated for 24 h then washed 3 × with PBS and aspirated. Migrated monocyte-enriched PBMCs on the membrane were counted. Incubation times and solute concentrations used in these experiments were selected based on manufacturer suggestions and preliminary data (not shown).

2.3. B cell enrichment and migration assay

PBMCs were thawed as seen in 2.3. B-cells were isolated with EasySep Human B Cell $CD19^+$ Positive Selection Kit (Stemcell). 0.6 mL of RPMI containing 20 ng/mL C-X-C motif ligand 13 (CSCL13) (eBioscience) was added in the bottom chamber of 5 μm pore transwell plates and left to equilibrate for 90 min as in section 2.2. Null controls received no CXCL13. B cells (0.1 mL at 1×10^6/mL) suspended in RPMI containing 100 μg/mL of LA were added to each top chamber Control aliquots (Null, UNT and LPS) contained no LA. After the 10 min pre-treatment period, top chambers were stimulated with 1 μg/mL LPS. Control aliquots (Null, UNT and LA) were not stimulated with LPS. After an 18 h incubation period as before, samples were collected from the bottom chamber and cells were counted. Incubation times and solute concentrations used in these experiments were selected based on manufacturer suggestions and preliminary data (not shown).

2.4. Data and statistical analyses

Samples were coded with a three digit identifier, blinding the research team from donor identification, disease state, and treatment conditions. In the monocyte migration assays, each sample was counted 5 × in random fields of view at 200 × magnification and averages and standard errors of the mean (SEM) were calculated. In B cell migration assays, cells were collected and counted in triplicate using a hemocytometer and averages ± SEM were calculated. All conditions were compared using Tukey's boxplots, two-way paired student *t*-tests and ANOVA. Values were considered significant if $p \leq 0.05$.

Table 1
Subject demographics.

	Healthy control	Relapsing remitting MS	
Number of subjects[a]	10	9	
%Male	80%	89%	p = 0.620
Age (Years)			
Average ± SEM	41.9 ± 3.63	41.6 ± 3.73	p = 0.951
Median	40	36	
Range	27–61	29–62	
Race			
% Non-hispanic Caucasian	80%	66.7%	p = 0.537
Disease duration (Years)			
Average ± SEM	N/a	8.2 ± 1.92	
Median	N/a	6	
Range	N/a	2–19	
EDSS score			
Average ± SEM	N/a	3.1 ± 0.53	
Median	N/a	2	
Range	N/a	2–6	

[a] Not all subjects' blood samples yielded enough PBMCs to complete B cell assays; specific N for B cell assays are thus given in the figure legend.

3. Results and discussion

3.1. Subject demographics

The subject demographics are recorded in Table 1. Because the study recruitment was limited to veterans of the United States Armed Forces, our study is disproportionately male (80%) compared to most RRMS studies. This is atypical because RRMS is around three times more prevalent in females than males (Harbo et al., 2013). There were no significant differences in age or gender between HC and RRMS subjects.

3.2. LA treatment inhibits monocyte migration

In this study, CCL2 was used as the monocyte migration assay chemoattractant since there is evidence that it is the primary chemokine in recruiting monocytes to the sites of inflammation, via CCR2 receptor binding. In murine EAE models, a deficiency in CCL2 or CCR2 reduces disease severity (Mahad and Ransohoff, 2003). We found that basal monocyte migration was significantly greater in RRMS subjects compared to HC (Fig. 1. HC UNT vs RRMS UNT). This finding is consistent with a previous report showing that the expression of CCR2 receptor was significantly increased in MS populations in both major monocyte subtypes ($CD16^+$ and $CD\ 14^+$) (Chuluundorj et al., 2014). Taken together with our previous finding that monocytes from RRMS patients have an increased production of pro-inflammatory cytokines is suggestive of a more pro-inflammatory phenotype (Fiedler et al., 2017).

We also showed that treating monocytes with LA significantly decreased CCL2 induced migration compared to untreated RRMS and HC controls by 19% and 14% respectively (Fig. 1. LA Treament vs UNT, $p < 0.001$). There was no difference in percent change in migration between RRMS and HC monocytes ($p > 0.7$) suggesting that LA is equally effective at inhibiting migration in both groups. This is in agreement with reports by Marracci et al. showing that LA affected murine T cell migration into the CNS, effectively suppressing symptoms and treating mice with EAE (Marracci et al., 2002). Furthermore, in the