Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 3 (2016) 7-17

Blood-Based Biomarkers

MicroRNA deregulation and chemotaxis and phagocytosis impairment in Alzheimer's disease

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Abstract

Introduction: Mononuclear phagocytes play a critical role during Alzheimer's disease (AD) pathogenesis due to their contribution to innate immune responses and amyloid beta (Aβ) clearance mechanisms.
Methods: Blood-derived monocytes (BDMs) and monocyte-derived macrophages (MDMs) were isolated from blood of AD, mild cognitive impairment (MCI) patients, and age-matched healthy controls for molecular and phenotypic comparisons.
Results: The chemokine/chemokine receptor CCL2/CCR2 axis was impaired in BDMs from AD and MCI patients, causing a deficit in cell migration. Changes were also observed in MDM-mediated phagocytosis of Aβ fibrils, correlating with alterations in the expression and processing of the triggering receptor expressed on myeloid cells 2 (TREM2). Finally, immune-related microRNAs (miRNAs), including miR-155, -154, -200b, -27b, and -128, were found to be differentially expressed in these cells.
Discussion: This work provides evidence that chemotaxis and phagocytosis, two crucial innate immune functions, are impaired in AD and MCI patients. Correlations with miRNA levels suggest an epigenetic contribution to systemic immune dysfunction in AD.

Keywords: Alzheimer's disease; Monocytes; Macrophages; Chemotaxis; Phagocytosis; CCR2; TREM2; miRNAs

1. Background

Blood-derived monocytes (BDMs) have been shown to have a beneficial role in Alzheimer's disease (AD) mouse models, associated with a higher ability to clear amyloid beta (Aβ) deposits in the brain [1–3], when compared with resident microglia. This contribution depends on the expression of chemokine receptors, such as CCR2, which mediate BDM migration and infiltration into the brain parenchyma [4,5] and phagocytosis-related proteins, such as the new AD risk protein, triggering receptor expressed on myeloid cells 2 (TREM2) [6]. However, in humans, the role of BDMs and monocyte-derived macrophages (MDMs; which directly differentiate from BDMs within tissues) in AD is poorly explored. *In vitro*, macrophages of AD patients usually show minimal surface uptake and poor internalization of Aβ [7], although the ability of their blood monocyte precursors to infiltrate the AD brain is yet poorly studied.

The authors have declared that no conflict of interest exists.

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http://dx.doi.org/10.1016/j.dadm.2015.11.004

Both CCR2 and TREM2 have been directly implicated in AD pathology in different mouse models. Deficiency in CCR2 in the Tg2576 and APP_{Swe}/PS1 mice exacerbated amyloidosis [4,8], whereas transplantation of CCR2-competent cells into APP_{Swe}/PS1/$CCR2^{-/-}$ restored cognitive functions [5]. Moreover, two very recent studies implicated TREM2 in Aβ clearance *in vivo*, with contradictory results. Although 5XFAD mice deficient in TREM2 showed increased accumulation of Aβ and a decrease in the number of microglia around plaques [9], in the APPPS1 mouse model, the absence of TREM2 resulted in a reduction of Aβ load [10]. In contrast with previous reports that restricted TREM2 expression to microglia cells [11], the study in the APPPS1 model hypothesizes that the $TREM2^{+}$ cells found to surround Aβ plaques are blood-derived macrophages. Despite these evidences, it is unknown whether, in the context of human disease, these cells are able to migrate to the AD brain and phagocyte Aβ and how their function is regulated at the molecular level.

We have recently reported that overexpression of miR-155 occurs both in M1-activated microglia [12] and in the brain of 3xTg AD mice [13] and is critical for the establishment of a chronic inflammatory phenotype. In this study, we decided to further explore the ability of microRNAs (miRNAs) to control immune-specific phenotypes, which hints at a potential use as early biomarkers of neuroinflammation [14]. For this purpose, we compared the expression of immune-related miRNAs in AD and mild cognitive impairment (MCI) patients, with that in healthy age-matched control subjects. Deregulation of miRNA expression and functional impairments in chemotaxis and phagocytosis observed in AD and MCI patients were correlated with the levels of emergent proteins in the realm of AD research. This work suggests that the deregulation of specific immune-related miRNAs in AD patients may contribute to the observed dysfunctions in chemotaxis and phagocytosis.

2. Methods

2.1. Patient selection

Subjects (n = 124) were recruited at the Neurology Department, Coimbra University Hospital, 36 age-matched healthy controls (controls), 52 MCI patients (MCI), and 36 AD patients (AD), and were representative of the Portuguese Caucasian population. Patients' diagnostic investigation comprehended a standard clinical evaluation, routine laboratory tests and imaging studies (computed tomography [CT] or MRI), SPECT, and *APOE* allele genotyping. TREM2 genotyping for R47H mutation in exon 2 (associated with higher AD risk [15]) was performed in all AD and MCI patients where TREM2 expression was assessed. Positron emission tomography, cerebrospinal fluid analysis, and genetic studies were more restricted, although considered in younger patients.

A comprehensive cognitive-functional-psychological assessment battery was carried out by a team of neuropsychologists, following a standard protocol, and comprising several tests and scales: (1) cognitive instruments as the mini-mental state examination (MMSE) [16], the Montreal Cognitive Assessment (MoCA) [17], the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog) [18], and a comprehensive neuropsychological battery validated for the Portuguese population (Battery of Lisbon for the Assessment of Dementia; [19]) were used to explore memory and other cognitive domains; (2) the clinical dementia rating (CDR; [20]) was used for global staging; and (3) the geriatric depression scale (GDS-30; [21]) was used to exclude major depression. All MCI patients were classified at the global CDR staging of 0.5 (no functional impairment) and were selected according to Albert's [22] and Petersen's criteria [23]. The standard criteria for the diagnosis of AD patients were the Diagnostic and Statistical Manual of Mental Disorders–fourth edition and the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders [24]. The AD group included patients with mild disease (CDR = 1) or moderate to severe (CDR = 2 and 3). The control group comprised 36 cognitively healthy adults belonging to the local community (recruited among the patients' spouses, hospital or university staff, or their relatives), that were age, education, and gender matched to the patients. Controls had normal MMSE scores (>24) and were fully autonomous in daily life activities (CDR) according to the information obtained through a general practitioner, and/or an informant. Moreover, to be eligible for this study, subjects (patients and controls) should be in a stable condition, without acute significant events or recent/undergoing changes in medication. Exclusion criteria were (1) significant motor, visual or auditory deficits which could influence the neuropsychological performance; (2) neurologic/psychiatric conditions other than MCI or AD; (3) CT or MRI demonstration of significant vascular burden [25]; (4) diagnosis of diabetes, chronic inflammatory, neoplastic diseases, or prescription of anti-inflammatory drugs; (5) major depression indicated by a GDS score of 20 or more points; (6) active smokers; and (7) patients experiencing uncontrolled hypertension. Informed consent was obtained from all participants, and the study was conducted in accordance with the tenets of the Declaration of Helsinki with the approval of the local ethics committee. Subjects' information/classification was only disclosed in the end of the study.

2.2. Isolation of BDMs

For each study subject, a total of 20 mL of blood was collected in sterile EDTA-coated tubes. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. BDM isolation was performed using magnetic separation, employing CD14 MicroBeads