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INCREASED EXPRESSION OF TREM2 IN PERIPHERAL CELLS FROM MILD COGNITIVE IMPAIRMENT PATIENTS THAT PROGRESS INTO ALZHEIMER'S DISEASE

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Abstract

Background. Neuroinflammation plays a role in the etiopathogenesis of Alzheimer's disease (AD). TREM2, a cell surface receptor of the immunoglobulin superfamily, seems to have protective anti-inflammatory activity in AD.

Methods. We analyzed TREM2 expression in peripheral blood mononuclear cells from healthy subjects (CT) and from patients with either AD or mild cognitive impairment (MCI). MCI were re-evaluated at a two-year follow-up to investigate their progression to AD (MCI-AD), or lack thereof (MCI-MCI).

Results. TREM2 gene expression was higher in AD than CT, but was highest in MCI. At recruitment TREM2 levels were higher in MCI-AD than in MCI-MCI, and in MCI-AD were higher initially than at follow-up. TREM2 displayed a moderate degree of sensitivity and specificity for identifying MCI-AD in all MCI patients.

Our data showed higher TREM2 levels in ApoE ε4 carriers than non-carriers in MCI and particularly in MCI-AD.

Conclusions. These data seem to confirm the protective role of TREM2 in the preclinical stage of AD. Up-regulation of TREM2 in MCI-AD could be a mechanism to counteract the activation of neuroinflammatory processes. We speculate that TREM2 and ApoE ε4 may interact synergistically in the preclinical stage of AD. Therefore, TREM2 may be useful as an early peripheral biomarker for the development of AD.

Introduction

Triggering receptor expressed on myeloid cells 2 (TREM2) is a cell surface receptor belonging to the immunoglobulin superfamily that is expressed on myeloid cells, including monocytes, dendritic cells, osteoclasts, macrophages, microglia [1], and blood cells [2]. TREM2 is capable of binding gram-positive and gram-negative bacteria [3], as well as apoptotic neuron and nucleic acids released from dying cells [4].

TREM2 is subjected to proteolytic intramembranous cleavage and is released into the extracellular space as a soluble variant (sTREM2) [5]. sTREM2 can be detected in cerebrospinal fluid (CSF), plasma, and serum [6].

TREM2 promotes survival, proliferation, and remodelling of the actin cytoskeleton, which controls adhesion and migration [7]. TREM2 regulates key signalling events involved in the immune response and in the phagocytic activity of microglia [8], characterized by anti-inflammatory activity [9-11].

Neuroinflammation is a common feature of neurodegenerative diseases [12]. In Alzheimer's disease (AD), the inflammatory process is likely promoted by the accumulation of protein aggregates and cell damage [13]. The neurodegeneration in AD has been attributed to the release of proinflammatory cytokines from brain resident cells and, although less consistently, from peripheral cells [14].

There is increasing evidence that inflammation plays a role in brain changes that precede AD, and several studies show an alteration of proinflammatory cytokines in the brains of mild cognitive impairment (MCI) patients [15]. MCI is defined as a cognitive decline greater than that expected for an individual's age and education level but that does not interfere with activities of daily life. MCI have been noted to develop AD at a higher rate than cognitively normal subjects [16].

Genome-wide association studies have identified a low-frequency variant of TREM2 as a genetic risk factor for late-onset AD [17]. This variant has an odds ratio comparable to allele ε4 of apolipoprotein E (ApoE) [18], which is the most common risk factor for both late- and early-onset AD [19]. An interaction between TREM2 and ApoE in vitro has been described [20], suggesting that ApoE may act as a ligand to stimulate microglial function through TREM2 [21].

Several studies have provided evidence suggesting that TREM2 plays a protective role in AD, inhibiting the inflammatory response, enhancing phagocytosis of apoptotic neurons [22], and promoting microglial functions in response to amyloid-β (Aβ) deposition [1]. The identification of peripheral biomarkers for an early diagnosis of AD is urgently needed [23]. In this study, we investigated the involvement of TREM2 in the preclinical stage of AD, evaluating the expression of TREM2 in peripheral blood mononuclear cells (PBMCs) from patients with MCI, AD, and from healthy subjects (CT). MCI were re-evaluated after two years to investigate their progression to AD investigating the expression of TREM2 at recruitment and at a two-year follow-up.

Materials and methods

All subjects were outpatients recruited from the Geriatric Unit of the Fondazione IRCCS Ca' Granda in Milan, Italy. The study involved 57 MCI, 50 AD, and 42 non-demented CT matched for gender and age. MCI subjects met the criteria outlined by Petersen [24], and AD patients fulfilled the DSM IV and NINCDS-ARDA criteria [25].

All participants were evaluated for medical history, physical and neurological examination and neurocognitive evaluation (Mini-Mental State Examination). AD diagnosis was confirmed by brain imaging (MRI and PET) and, when the subjects consented to lumbar puncture, by the assessment of Aβ, tau, and P-tau levels by ELISA (Innogenetics, Ghent, Belgium).

MCI were clinically re-evaluated after a two-year follow-up to investigate progression to AD: 13 MCI advanced (MCI-AD) and 44 MCI did not advance (MCI-MCI) to AD. CT were assessed to exclude the presence of neurological and cognitive disorders. None of the selected subjects showed any clinical signs of inflammation.

At recruitment (T0), blood samples from all subjects were collected between 8 and 9 a.m., following a 6-hour fast. For 7 out of 13 MCI-AD and 8 out of 44 MCI-MCI, blood was collected also at a two-year follow-up (T1). The local ethics committee approved this study, and informed consent was obtained from either patients or legal representatives.

Genomic DNA was extracted from whole blood [26] and the ApoE genotype was determined [27].

PBMCs were collected from whole blood by using a density gradient centrifugation procedure (Lympholyte-H Cedarlane Laboratories Limited, ON).

Total RNA was extracted from PBMCs [28]. Quantitative relative PCR was performed using an ABI 7500 system (Applied Biosystem, CA). Specific TaqMan probes were employed for TREM2 (Life Tecnologies, Hs00219132_m1), and the relative levels of TREM2 expression were determined by comparing those of the housekeeping gene GAPDH (Life Tecnologies, Hs03929097_g1).

sTREM2 plasma levels were evaluated using the Human TREM2 ELISA kit (Sigma-Aldrich, USA).

Statistical analysis was performed using the SPSS statistical package (SPSS version 24, Chicago, IL). Data were expressed as mean values±standard error. Differences in TREM2 expression were evaluated by the Kruskal-Wallis, Mann-Whitney U, or Wilcoxon test. The predictive efficacy of TREM2 was assessed using the area under the curve (AUC) generated by a receiver operating characteristic (ROC) analysis. ApoE ε4 allelic distribution was

assessed using the χ^2-test. We adopted $p<0.05$ as the threshold value for statistical significance.

Results

All groups had a similar mean age. At recruitment MCI, AD and CT had significantly different MMSE scores (Table 1).

Table 1

Participants' characteristics.

When MCI patients were categorized based on their progression to AD, MCI-AD and MCI-MCI groups had similar MMSE scores at T0. The MMSE scores were significantly lower in MCI-AD than MCI-MCI at T1 (Table 2).

Table 2

MCI patients' characteristics.

At recruitment, our analysis showed significantly different TREM2 mRNA levels in PBMCs from CT, MCI and AD (0.66±.04, 0.91±0.06 and 0.89±0.07, respectively; $p<0.05$). TREM2 expression was significantly higher for both the MCI ($p<0.001$) and AD groups ($p<0.05$) compared to CT (Fig.1).

Fig.1 TREM2 mRNA levels in CT, MCI, and AD. *p<0.05 vs CT.

At T0 TREM2 gene expression was significantly higher in MCI-AD compared to MCI-MCI (1.17±0.14 and 0.83±0.06; p<0.05), AD (p<0.05), and CT (p<0.001). TREM2 mRNA levels were higher in MCI-MCI than CT (p<0.05) (Fig.2A).

MCI-AD showed significantly higher TREM2 gene expression at T0 than T1 (1.21±0.2 and 0.91±0.2; p<0.05). Levels were similar in MCI-MCI both at T0 and T1 (0.71±0.1 and 0.65±0.1) (Fig.2B).

Fig.2 [A] TREM2 mRNA levels in CT, MCI-AD, MCI-MCI and AD and [B] from MCI-AD and MCI-MCI at T0 and at T1. *p<0.05 vs CT; **p<0.05 vs MCI-MCI, AD and CT; ***p<0.05 vs T1 MCI-AD.

ROC analysis shows that TREM2 gene expression at T0 is able to identify MCI-AD from a heterogeneous group of MCI patients with AUC 0.69 (95% CI, 0.53–0.84).

Despite there being a similar trend for plasma sTREM2 levels and TREM2 gene expression levels, differences among groups for sTREM2 levels were not statistically significant (data not shown).

We found a different distribution of ApoE ε4 carriers among the three groups: 32.7% of MCI, 56.8% of AD, and 26.2% of CT were carriers (p<0.05). The MCI-AD had a higher number of ε4 carriers (53.8%) than the MCI-MCI (26.7%).

ApoE ε4 allele carriers showed a higher TREM2 gene expression than non-carriers (0.96±0.07 and 0.75±0.03; p<0.05), regardless of diagnosis (Fig.3A). Significantly different TREM2 levels were found between ε4 carriers and non-carriers in all MCI (1.08±0.11 and 0.82±0.06; p<0.05), but not in AD (0.99±0.10 and 0.75±0.08) or CT (0.66±0.05 and

0.68±0.10) (Fig.3B). MCI-AD ε4 allele carriers had higher TREM2 levels than non-carriers (1.37±0.2 and 0.93±0.15; $p<0.05$). Conversely, MCI-MCI ε4 carriers and non-carriers showed very similar results (0.92±0.12 and 0.80±0.06) (Fig.3C).

Fig.3 TREM2 mRNA levels in ApoE ε4 allele carriers (ε4+) and non-carriers (ε4-) subjects: [A] regardless of diagnosis; [B] in CT, MCI and AD; [C] in MCI-AD and MCI-MCI. *$p<0.05$ vs ε4-; **$p<0.05$ vs MCI ε4-; ***$p<0.05$ vs MCI-AD ε4-.

Discussion

In AD pathology, the persistent activation of microglia and chronic neuroinflammation could have detrimental effects on brain function and promote neurodegeneration. TREM2 might protect against neuroinflammation by inhibiting the persistent activation of microglia, promoting phagocytosis, and clearing apoptotic neurons [1].

In this preliminary study, we analyzed PBMCs since these cells seem to be modified in several neurological diseases [29-31]. Many studies have confirmed that PBMCs likely provide a window into the CNS, and that they may be a useful model to better understand the mechanisms altered in tissue not easily accessible [29].

Controversial data exist about TREM2 expression in circulating cells. Hu et al. argue that TREM2 is not expressed in lymphocytes [32]. Instead, recently, TREM2 mRNA expression has been described in whole blood [33, 34]. In particular, higher levels of TREM2 mRNA were detected in whole blood from AD compared to amnestic MCI patients and healthy subjects [34].

Our data confirm that TREM2 is expressed in blood and, in particular, in PBMCs. Unlike Tan et al., we found the highest levels not only in AD, but also in MCI.

The percentage of our MCI patients progressed to AD within two years (22.8%) is in line with the data showing an annual rate of conversion of 10-15% in clinical setting [35].

Albeit the reduced number of MCI-AD, our study is the first that evaluated TREM2 gene expression in a perspective setting, analysing the same patient before and after the progression.

Interestingly, we observed that, at recruitment, MCI who progressed to AD within two years showed a higher TREM2 gene expression than MCI who did not develop AD. Moreover, in MCI-AD, TREM2 gene expression was significantly higher at recruitment than two years later. This difference was not present in MCI-MCI group.

These data support the hypothesis that TREM2 plays a protective role in the preclinical stage of AD pathology. Accordingly, we speculate that the up-regulation of TREM2 could be a mechanism to counteract neuroinflammatory processes in MCI patients who progress to AD. Indeed, the MCI-MCI group included MCI patients with different neuropathological characteristics who did not, and may never, progress to AD.

The protective role of TREM2 could be prominent in triggering neuroinflammation. Indeed, we found the highest TREM2 levels during the early phase of neuroinflammation. Conversely, when the inflammatory process becomes chronic in overt AD, TREM2 expression decreases.

Previously, our group had focused on the role of molecules involved in the modulation of inflammation in the preclinical stages of AD. We have observed an up-regulation of adenosine receptors in PBMCs from MCI patients, confirming that there is an altered expression of receptors involved in countering the inflammatory processes, and that this alteration appears before the onset of the clinical symptoms of AD [36].

ApoE has been identified as an agonist for TREM2 [21], suggesting that apoE-TREM2 interaction in microglia could play a role in the modulation of the phagocytosis of apoE-

bound apoptotic neurons, in the enhancement of the Aβ clearance, and in the regulation of TREM2 mediated signalling in microglia.

We found higher TREM2 mRNA levels in ApoE ε4 carriers than non-carriers, regardless of the diagnosis. Our data showed higher TREM2 levels in ApoE ε4 carriers than non-carriers in all MCI, but not in AD or CT. Moreover, when categorizing MCI by progression to AD, we found higher TREM2 levels in ApoE ε4 carriers than non-carriers in MCI-AD but not in MCI-MCI. Our results may suggest that TREM2 and ApoE ε4 interact synergistically in the preclinical stage of AD, particularly in MCI patients who advance to AD.

Analyzing plasma sTREM2, differences among groups were not statistically significant.

A significant increase of CSF sTREM2 levels has been described in the early symptomatic phase of AD [37] and in mild dementia [38]. sTREM2 levels in CSF not correlated with sTREM2 in plasma supporting the hypothesis that CSF sTREM2 is produced in situ [38]. Our study seems to confirm these data.

Further studies are needed to analyze not only the soluble form of TREM2, but also the protein expressed on the cell surface.

Early diagnosis and identification of preclinical AD are particularly important issues. There is a need for a reliable, minimally invasive, and inexpensive biomarker for dementia, which has lead many to investigate various components of peripheral blood.

According to the ROC curve analysis, TREM2 displays a moderate degree of sensitivity and specificity for identifying which MCI will progress to AD within two years, suggesting that TREM2 could be a good candidate as a peripheral and early biomarker for AD.

Conflict of interest

The authors have no conflicts of interests.

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	CT	MCI	AD	p-value
n° of partecipants	42	57	50	
Age	78.9±0.7	78.5±0.8	78.6±0.6	N.S.
MMSE score T0	29.1±0.2	26.2±0.4	21.9±0.6	<0.001

	MCI-AD	MCI-MCI	p-value
n° of partecipants	13	44	
Age	75.1±1.3	79.4±0.9	N.S.
MMSE score T0	26.6±0.6	26.1±0.4	N.S.
MMSE score T1	22.6±1.5	25.31±0.6	0.04