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Short Communication

Increased expression of Interleukin-18 receptor in blood cells of subjects with Mild Cognitive Impairment and Alzheimer's disease

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ABSTRACT

Inflammation has been proposed as a leading force in neurodegeneration and Interleukin (IL)-18 is a pro-inflammatory cytokine which is suggested to be implicated in Alzheimer's disease (AD). However, the meaning of the IL-18 participation in this disease is still unclear.

Since IL-18 activity is mediated by its heterodimeric receptor complex IL-18Rα/β, we evaluated the presence of both IL-18R chains on peripheral blood cells of AD patients, as well as in individuals with Mild Cognitive Impairment (MCI), at increased risk to develop AD. More specifically, we compared the levels of $CD14^+$ monocytes and $CD3^+$ T-lymphocytes bearing IL-18Rα and β chains in the two groups of patients with those in healthy control subjects, both before and after *in vitro* cell treatment with lipopolysaccharide (LPS).

While no differences in the levels of monocytes and T-lymphocytes bearing IL-18Rα chain were found among the three groups, either in untreated and LPS-treated conditions, the IL-18Rβ chain expression appeared differently regulated in MCI and AD patients, as compared to controls. In particular, the amount of IL-18Rβ-bearing monocytes was similar among the three groups at unstimulated conditions, while after LPS treatment it was increased in MCI *vs.* controls. A significant increase of IL-18Rβ-bearing T-lymphocytes was also observed in MCI and AD *vs.* controls, both in untreated and LPS-stimulated conditions.

Our findings indicate that the expression of IL-18R complex on blood cells is perturbed in AD and even more markedly in its preclinical state of MCI, confirming that an increased peripheral activity of IL-18 may be involved in the early phase of AD pathophysiology.

1. Introduction

The brain of AD patients is characterized by the presence of activated microglia cells surrounding the amyloid plaques and releasing pro-inflammatory cytokines, which likely contribute to disease's progression and chronicity [1]. Interestingly, the neuroinflammatory response activation might occur in the early stages of AD pathology [2]. Albeit inflammation-related brain damage is postulated to be mainly due to activation of resident microglia, neurodegenerative changes in the brain appear to be also associated with changes in the peripheral immune system [3,4]. Among the several pro-inflammatory factors assumed to participate in AD neurodegeneration, the IL-1 related cytokine IL-18 has been associated with AD at multiple levels, although its exact role in AD needs still to be clarified. In particular, some variants of IL-18 gene promoter correlating with AD risk and outcome have been described, and at protein level, an increased expression in the brain and elevated peripheral amounts of IL-18 in blood cells of AD patients, correlating with cognitive impairment, have been also reported [5]. IL-18 acts through the binding to its specific receptor complex (IL-18R), consisting of a binding α chain and a signaling β chain, which are both necessary for having a functional receptor [6]. In order to better understand the IL-18 biology in AD development, the study of IL-18R complex should be taken into account, but no studies have been so far addressed to this subject. Thus, we analyzed here the percentage of cells bearing the IL-18R chains within unstimulated or LPS-stimulated monocytes and T-lymphocytes obtained from blood of AD patients and individuals suffering from Mild Cognitive Impairment (MCI), which could develop AD [7], in comparison to cells from age-matched healthy control subjects (HC).

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2. Materials and methods

2.1. Subjects

Thirteen patients with diagnosis of probable AD, 24 with Mild Cognitive Impairment (MCI) and 25 healthy control subjects (HC) were selected. Medical and psychiatric histories were obtained from each subject, and all patients underwent a series of standard clinical examinations, including physical, neurological and mental status examinations, neurocognitive tests, and brain magnetic resonance imaging.

Inclusion criteria for AD were: (1) diagnostic evidence of probable AD consistent with the NINCDS-ADRDA criteria; and (2) a MMSE score $\geqslant$15. Inclusion criteria for MCI were: (1) diagnostic evidence of amnestic MCI consistent with Petersen guidelines [7] and (2) a Mini Mental State Examination (MMSE) score $\geqslant$23. A specific inclusion criterion for HC required that all neuropsychological scores were above the cut-off scores identifying normal cognitive level. Common exclusion criteria were: (1) major medical illnesses and autoimmune-inflammatory diseases; (2) co-morbidity of primary psychiatric or neurological disorders and any other significant mental or neurological disorder; (3) MRI evidence of focal parenchymal abnormalities or neoplasm. Informed written consent was obtained from all subjects or, when necessary, from their proxies and the study was conducted in accordance with Declaration of Helsinki and Local Ethics Committee. Demographic and clinical characteristics of subjects included in the study are summarized in Table 1.

2.2. Cell preparation

Peripheral blood mononuclear cells (PBMC) from HC, MCI and AD subjects were obtained after Ficoll-Hypaque gradient centrifugation of fresh blood and analyzed after culture, as indicated. The culture conditions of PBMC were selected on the basis of our previous study [8]. Briefly, two millions of PBMC were cultured in 48-wells culture plates in RPMI 1640 medium (Invitrogen Life Technologies) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and incubated at 37 °C, 5% CO_2 for 18 h in absence or presence of the pro-inflammatory stimulus lipopolysaccharide (LPS; 200 ng/ml; *E. coli* O55:B55, Sigma). Cell viability, as measured by trypan blue exclusion, was greater than 96% in all culture conditions.

2.3. Flow cytometry analysis

Untreated and LPS-treated PBMC were collected, washed with PBS and incubated for 15 min at 4 °C with fluorescent dye-labelled monoclonal antibodies anti-CD3-APC (e-Bioscience), anti-CD14-PerCP (BD Biosciences), anti-IL18Rα-PE and anti-IL-18Rβ-PE (R&D Systems) and washed again. Fluorescence emission of cell suspensions was monitored by a FACSCalibur flow cytometer (BD Immunocytometry Systems). In order to exclude debris and to measure the presence of IL-18R chains on selected PBMC subpopulations, monocytes and lymphocytes were gated based on Side Scatter (SSC) and Forward Scatter (FSC) parameters. Within monocyte and lymphocyte gates, $CD14^+$ and $CD3^+$ cells, respectively, were evaluated for the binding of antibodies specific for IL-18Rα or IL-18Rβ. Thus, data of IL-18R chain frequency are expressed as percentage of double positive cells for both CD14 or CD3 and IL-18Rα or IL-18Rβ in each PBMC subpopulation. Data were analyzed using BD CellQuest Pro version 6.0 Software (BD Biosciences) and herein reported as mean percentage of positive cells ± standard error (SEM).

$CD14^+$ monocytes and $CD3^+$ T-lymphocytes were equally represented among PBMC of the three groups of subjects, as evidenced in untreated and LPS-treated PBMC by percentage values (% mean ± SEM) of $CD14^+$ (untreated: HC = 10.9 ± 1.0, MCI = 11.0 ± 1.0, AD = 11.2 ± 1.3; LPS-treated: HC = 14.2 ± 1.2, MCI = 15.3 ± 1.6, AD = 11.8 ± 1.5) and $CD3^+$ cells (untreated: HC = 51.2 ± 2.4, MCI = 48.0 ± 2.4, AD = 47.5 ± 3.7; LPS-treated: HC = 51.4 ± 2.4, MCI = 51.1 ± 2.2, AD = 49.4 ± 4.7).

Table 1
Clinical characteristics of healthy controls, Mild Cognitive Impairment subjects and Alzheimer's disease patients.

	HC	MCI	AD
Total number	25	24	13
Age (years)	67.4 ± 1.4	71.0 ± 1.3	72.5 ± 2.3
Gender male (M/F)	14/11	14/10	6/7
Years of education	12.8 ± 0.8	8.1 ± 0.7*	7.3 ± 1.4**
MMSE score	29.1 ± 0.2	27.2 ± 0.4**	22.2 ± 0.9**,#

Data are expressed as mean ± standard error.
* $p < 0.01$ *vs.* Healthy subjects.
** $p < 0.001$ *vs.* Healthy subjects.
# $p < 0.001$ *vs.* MCI.

2.4. Statistical analysis

Since data were not normally distributed as assessed by the D'Agostino & Pearson omnibus normality test, statistical analyses were performed using non-parametric tests. The percentage of $CD14^+$ monocytes or $CD3^+$ T-lymphocytes positive for IL-18Rα or IL-18Rβ was compared among the groups (AD *vs.* HC and MCI *vs.* HC) using Mann–Whitney *U* test, in both unstimulated and LPS-stimulated conditions. Comparison of unstimulated *vs.* LPS-stimulated conditions within each group was evaluated using paired Wilcoxon *U* test. *p*-Values less than 0.05 were reported to be statistically significant. Statistical analyses were done using the Graph Pad Prism version 4.

3. Results

Unstimulated and LPS-stimulated PBMC derived from HC, MCI and AD subjects were analyzed as percentages of $CD14^+$ monocytes and $CD3^+$ T-lymphocytes expressing IL-18Rα or IL-18Rβ chain and results are reported in Fig. 1. Comparable percentage of IL-18Rα^+/$CD14^+$ cells within monocytes (Fig. 1, upper left panel) and IL-18Rα^+/$CD3^+$ within T-lymphocytes (Fig. 1, lower left panel), both at unstimulated and LPS-stimulated conditions were observed. Similarly, the percentage of untreated $CD14^+$ cells expressing IL-18Rβ was comparable among the three groups (Fig. 1, upper right panel). However, after LPS treatment, the percentage of IL-18Rβ^+/$CD14^+$ cells within monocyte population increased significantly in MCI as compared to HC ($p = 0.03$), while no differences were seen between AD and HC or AD and MCI cells (Fig. 1, upper right panel). When $CD3^+$ T-cells expressing IL-18Rβ were evaluated, a remarkable difference between HC and the two groups of patients was highlighted both at untreated and LPS-treated conditions (Fig. 1, lower right panel). In fact, a significantly increased amount of IL-18Rβ^+/$CD3^+$ cells was present in MCI and AD within lymphocyte cells at the untreated condition, as compared to HC subjects (MCI *vs.* HC: $p = 0.0004$; AD *vs.* HC: $p = 0.002$) and even more marked differences were found in the same cells following LPS treatment (MCI *vs.* HC: $p < 0.0001$; AD *vs.* HC: $p = 0.0002$) (Fig. 1, lower right panel).

Then, the ability of LPS to modulate the frequency of cells bearing the two IL-18R chains, as compared to unstimulated conditions, was evaluated in each group of subjects. Briefly, as observable in the figure, IL-18Rα^+ cells were significantly increased by LPS within