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Changes in Plasma β-NGF and Its Receptors Expression on Peripheral Blood Monocytes During Alzheimer's Disease Progression

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Abstract. Alzheimer's disease (AD), the most common cause of dementia, is characterized by the deposition of extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles, and by neuroinflammation. During the pathogenesis of AD, monocyte-macrophage lineage cells become increasingly ineffective in clearing Aβ deposits, less able to differentiate, and shift toward pro-inflammatory processes. Beta-nerve growth factor (β-NGF) and its receptors, TrKA and p75NTR, produce several biological responses, including cell apoptosis and survival, and inflammation. In the central nervous system, the involvement of these receptors in several critical hallmarks of AD is well known, but their role in circulating monocytes during the progression of dementia is unclear. We investigated the relationship between plasma β-NGF concentration and TrkA/p75NTR receptor expression in monocytes of patients with mild cognitive impairment (MCI), mild AD, and severe AD. We observed that plasma β-NGF concentration was increased with a higher expression of TrKA, but not of p75NTR, in monocytes from patients with MCI and mild AD, whereas β-NGF concentration and TrKA expression were decreased and p75NTR expression was increased, associated with caspase 3-mediated apoptosis, in patients with severe AD. In our study, we show evidence of variation in plasmatic β-NGF and monocytic TrkA/p75NTR receptor expression during the progression of dementia. These novel findings add evidence to support the hypothesis for the involvement of β-NGF and its receptors on monocytes during AD progression.

Keywords: Alzheimer's disease, β-NGF, mild cognitive impairment, monocytes, p75NTR, TrKA

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is associated with extracellular deposition of amyloid-β protein (Aβ), intracellular neurofibrillary tangles, and synaptic and neuronal loss [1]. Experimental results suggest that a coexistent increase in neuroinflammation and a decrease in Aβ degradation are relevant in AD pathogenesis and progression [2, 3] and that monocyte-macrophage lineage cells play an important biological role in this context. In AD, microglia cells, the resident macrophages of the brain, are activated to phagocytize and degrade Aβ peptide. Also, they secrete elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β, and tumor necrosis factor alpha (TNFα) [4]. Altogether, this pro-inflammatory milieu contributes to neuronal injury in the central nervous system (CNS) [5].

Accumulating evidence points to the pivotal role of peripheral monocytes in the pathogenesis of AD. These circulating phagocytes are recruited in microglia-damaged areas where they influence local brain inflammation [6–8]. In comparison with healthy subjects, peripheral monocytes of AD patients present complex *in vitro* and *in vivo* impairments: inefficient clearance of Aβ peptides, poor ability to differentiate, marked shortening of telomere length, and increased production of pro-inflammatory cytokines [9–13].

Conventionally, monocytes are divided into three different functional populations based on the expression of CD14 and CD16: classical monocytes ($CD14^{++}/CD16^{-}$) present anti-inflammatory characteristics, produce high levels of IL-10, and exhibit phagocytic activity; intermediate ($CD14^{++}/CD16^{+}$) and non-classical ($CD14^{+}/CD16^{++}$) monocytes have an inflammatory function, secret IL-1β and TNFα, and show low peroxidase activity [14].

Nerve growth factor (NGF) is a multifunctional neurotrophin that, besides its role in neuronal growth and survival, exerts pleiotropic effects on various populations of non-neuronal cells and is involved in cancer growth and inflammation [15–18]. β-NGF, the biologically active subunit of NGF, acts through two types of receptors: the specific high-affinity tropomyosin-related kinase A (TrKA) receptor and the common low-affinity p75 receptor for neurotrophins (p75NTR) that belongs to the TNF death receptor family [19]. TrkA receptors activate cell signaling pathways that induce cell proliferation, differentiation, and survival, while p75NTR has both pro- and anti-apoptotic effects [20, 21]. Monocytes, macrophages, and T- and B-lymphocytes normally express both of these types of β-NGF receptors [22].

Alterations in β-NGF metabolic pathways in the CNS have been linked to the development of AD. Several studies have demonstrated that extracellular maturation of the NGF precursor (proNGF) is impaired in AD, with enhanced degradation of β-NGF, followed by a decrease in TrKA and an increase in p75NTR expression in neurons [23, 24]. The peculiar increase in p75NTR expression has been associated with this receptor's ability to bind and internalize Aβ [25]. Furthermore, β-NGF-dependent basal forebrain cholinergic neurons are known to progressively degenerate during AD [26]. The increase in β-NGF and inflammatory cytokine concentrations near Aβ plaques appear to be one of the early molecular events that lead to neurodegeneration in AD [27, 28]. Analysis of cerebrospinal fluid (CSF) has also demonstrated that β-NGF concentration is higher in AD patients than in healthy controls [29–31]. Moreover, proNGF levels were found to be increased in patients with mild cognitive impairment (MCI) and AD as compared with healthy subjects and correlated with greater impairment on cognitive test scores [32].

Although data on the involvement of β-NGF and its receptors in the CNS during AD development are congruent and converging, evidence for their changes in peripheral blood cells are controversial. Schaub et al. [33] reported a decrease in serum β-NGF in pre-dementia, whereas Faria et al. [34] reported no difference in patients with MCI or AD as compared with healthy subjects.

The aim of this study was to investigate the relationship between plasma β-NGF concentration and TrkA/p75NTR receptor expression on monocytes from patients with MCI, mild AD, and severe AD in order to evaluate their peripheral involvement during the progression of dementia.

MATERIALS AND METHODS

Sample collection

Forty-six patients with MCI or AD were recruited at the Neuromotor and Cognitive Rehabilitation Research Center (Department of Neurosciences, Biomedicine and Movement Sciences), Azienda Ospedaliera Universitaria Integrata of Verona, and

at the Mons. A. Mazzali Foundation, Mantua, Italy. Clinical diagnosis of MCI and AD was established according to the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) work group criteria for probable AD [35]. Dementia severity was assessed by means of the Mini-Mental Status Examination (MMSE) [36] and the Clinical Dementia Rating scale (CDR) [37, 38]. The AD patients were divided in two groups according to the severity of dementia: those with MMSE scores <15 and CDR 3 were categorized as having severe AD (sAD), those with MMSE scores between 15 and 24 and CDR 1-2 were categorized as having mild AD (mAD), while those with MMSE scores >24 and CDR 0–0.5 were categorized as having MCI.

A control group (CTRL) composed of healthy elderly individuals was recruited from the same geographical area. All subjects underwent complete clinical and laboratory investigations. Exclusion criteria were: a history of depression or psychosis, alcohol or drug abuse, other neurological (e.g., multiple sclerosis, Parkinson's disease, brain injuries, stroke), cardiac, orthopedic (e.g., osteoarthrosis), or respiratory conditions (e.g., chronic obstructive pulmonary obstruction). All experiments with human samples were done after informed consent obtained from the patients or their relatives in accordance with the tenets of the Declaration of Helsinki, as part of a protocol approved by the Institutional Review Board of the Azienda Ospedaliera Universitaria Integrata (Verona, Italy).

Sample preparation

Venous peripheral blood (20 mL) was routinely collected between 9 : 00 and 10 : 00 from patients and CTRL in a fasted state and processed within 2 h to obtain plasma and peripheral blood mononuclear cells (PBMCs).

Plasma preparation

Plasma was separated from peripheral blood by centrifugation (1200 rpm for 20 min at 4°C) and kept at –80°C until analysis [39].

Isolation of human PBMCs and monocytes

Blood was drawn on ethylenediaminetetraacetic (EDTA) acid and diluted in phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 2 mM EDTA, pH 7.4 (PBS/EDTA) and layered on Lympholyte® (Cedarlane Laboratories Limited, Burlington, ON, Canada) (density 1.077 g/mL) according to the manufacturer's protocol. The resulting interphase containing PBMCs was isolated and washed with PBS/EDTA at 300 *g* for 10 min at 21°C. Platelets were removed by repeated centrifugation at 200 *g* for 15 min.

Monocytes were isolated from PBMCs by depletion of $CD14^-$ cells (negative selection) using a Monocyte Isolation Kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purity of monocytes was more than 96% as determined by a fluorescent-activated cell sorter using anti-CD14 antibody (Miltenyi Biotec).

β-NGF determination

Plasma and medium concentration of β-NGF were measured by enzyme immunoassay ELISA (DuoSet ELISA development R&D System, #DY256-05) according to the manufacturer's protocol. The amount of β-NGF was determined by measuring absorbance at 450 nm. The standard curve demonstrated a direct relationship between optical density and β-NGF concentration. Plasma or culture medium β-NGF concentration was expressed in pg/mL (detection limit 31.25 pg/mL) or pg/10^5 cells, respectively. Though the kit is designed to measure mature β-NGF, we cannot exclude that it may also be used to measure pro-NGF.

Flow cytometry immunofluorescent staining

Freshly purified PBMCs and cells cultured in the presence/absence of $A\beta_{42}$ either alone or with β-NGF were resuspended in PBS containing 0.5% BSA (Gibco BRL, Milan, Italy) at a final concentration of 1×10^6 cells/mL. All procedures were performed at 4°C. A gating strategy was used for FACScan analysis. In brief, cells were visualized first on a forward and side light scatter plot, and a gate was drawn around the monocyte population while excluding the remaining contaminating lymphocytes, neutrophils, and debris. Monocytes and their subsets were concurrently identified through stepwise gating of daughter populations [40]. Neutrophils, natural killer (NK) and natural killer T (NKT) cells were excluded from these cells by CD66b expression, and then CD56 and CD3 expression, respectively. The resultant population

was then visualized on a plot comparing CD14 and CD16, and a gate was drawn to define the classical banana-shaped monocyte cloud. The gates were then placed around the monocyte subsets. The following fluorochrome-labeled mAbs were used to identify TrKA and p75NTR receptors on monocyte subsets: FITC-labeled rabbit anti-human p75NTR extracellular domain (#ANT-007-F, Alomone Labs, Jerusalem, Israel) [41] and PE-labeled mouse anti-human TrKA (FAB1751P, R&D System, Italy) [42]. The following Abs were used for the gating strategy and monocyte immunophenotyping: PerCP-Cy5.5 conjugated anti-CD14 mAb (#550787, clone M5E2, Becton Dickinson, Franklin Lakes, NJ, USA), mAb FITC-conjugated anti-CD66 mAb (#555724, clone G10F5, Becton Dickinson), PE-conjugated anti-CD56 (#555516, clone B159, Becton Dickinson), PE-conjugated anti-CD3 (#555340, clone HIT3a, Becton Dickinson), and PE- and FITC-conjugated anti-CD16 mAb (#555407 and #555406 respectively, Becton Dickinson). All FACS reagents were used at the concentration recommended by the manufacturers. Cells were then washed, resuspended in staining buffer (PBS+2% FBS+1% paraformaldehyde), and analyzed by FACS or analyzed for intracellular cytokine staining.

Intracellular cytokine staining

Intracellular staining was performed after surface specific antibody labeling as described above. Briefly, cells were treated with Perm/Fix solution (FIX&PERM Cell Permeabilization kits, #88-8824-00, eBioscience) and incubated for 30 min at 4°C in the dark with IL6-PE-conjugated (eBioscience, #12-7069) and IL10-PE-conjugated (eBioscience, #12-0167). After washing with Perm/Fix solution, cells were resuspended in staining buffer and processed by flow cytometry analysis.

Flow cytometry analysis

A FACS Calibur (BD) equipped with a single 15-mW argon ion laser operating at 488 and 633 nm and interfaced with CellQuest Software (Becton Dickinson) was used. Samples were run using isotype controls or single fluorochrome-stained preparations for color compensation. The results were expressed as percentage of positive cells/antibody used for staining (% positive cells) and as mean fluorescent intensity (MFI).

Real time RT PCR

Total RNA of monocytes was isolated using an RNeasy Mini Kit (Qiagen). Reverse transcription was carried out using 1 μg of total RNA in the presence of 200 U of SuperScript® II Reverse Transcriptase U and random hexamers (Invitrogen). Real time RT-PCR assays were performed using 5 ng total RNA converted to cDNA and primers for TrKA (NTRK1, 5′-CAGCCGGCACCGTCTCT-3′ 5′-TCCAGGAAC TCAGTGAAGATGAAG-3′) and p75NTR (NGFR, 5′-GAGGCACCTCCAGAACAAGA-3′ 5′-GCTGT TCCACCTCTTGAAGG-3′) genes (Sigma and Brilliant® II SYBR® Green QPCR Master Mix, Agilent Technologies). A StepOne System (Applied Biosystems) was used to collect the signals. Relative quantification of gene expression was determined using GAPDH (5′-TCGTGGAAGGACTCATGA CC-3′ and 5′-TCAGCTCAGGGATGACCTTG-3′) as endogenous control [43].

Cell treatment with Aβ peptide and β-NGF

Freshly purified peripheral blood monocytes isolated from healthy donors healthy donors, MCI, mAD, and sAD patients were incubated for 24 h with 10 μg/mL of $A\beta_{42}$ peptide (AnaSpec Inc, AS-24224) in RPMI 1640 culture medium supplemented with 10% FCS, 2 mM L-glutamine, and 100 U/mL Pen/Strep (Invitrogen) at 1×10^6 cells/mL. In separate experiments, monocytes from the healthy donors were treated for 24 h with 100 ng/mL of β-NGF (Alomone, Israel) either alone or together with 10 μg/mL of $A\beta_{42}$. In both experimental conditions, cell culture mediums were collected after 24 h and analyzed by ELISA to detect β-NGF levels. At the same time, monocytes were analyzed by FACS for CD14 and CD16, TrKA/p75NTR expression, and intracellular cytokine levels. Moreover, sub-G1 cell cycle distribution and activation of caspase-3 were evaluated under these experimental conditions.

FACS analysis of sub-G1 phase distribution

FACS analysis was used to determine the sub-G1 fraction (dead cells) by propidium iodide (PI). Monocytes isolated from CTRL, MCI, mAD, and sAD subjects were cultured in the absence/presence of $A\beta_{42}$, as previously described. Cells were harvested after 24 h, washed with PBS, and fixed in 5 mL of 70% cold ethanol for at least 2 h at 4°C. After washing with PBS, the cells were treated with RNase A

(250 μg/mL) and PI (50 μg/mL) in PBS plus 0.2% FBS at 37°C for 30–45 min and analyzed by flow cytometry.

Cleaved caspase 3 ELISA kit

Cleaved caspase-3 was quantified using a PathScan Cleaved Caspase-3 (Asp175) sandwich ELISA kit (Cell Signaling, #7190,) according to the manufacturer's recommendations.

Statistical analysis

Results are expressed as means ± standard deviation (SD). Comparison of the two experimental groups was done using Student's *t*-test. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison tests. The correlation between variables was analyzed using Pearson's correlation coefficient (r) and the coefficient of determination (r^2). Statistical significance was set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism 5 software.

RESULTS

Patients

Table 1 presents the clinical and demographic characteristics of the 59 subjects: patients with MCI (n = 10), mAD (n = 19), sAD (n = 17), and age-matched controls (CTRL, n = 13). The four groups were homogeneous for age, except for the sAD patients who were significantly older than the CTRL ($^{**}p < 0.001$) (CTRL = 76 ± 4, MCI = 73.8 ± 2.9, mAD = 77.6 ± 6.5, and sAD = 85.62 ± 6.2 years old), gender (M/F; CTRL = 5/8, MCI = 6/4, mAD = 7/12, sAD = 10/7), and education (CTRL = 6.3 ± 2, MCI = 8.6 ± 2.6. mAD = 7.36 ± 4.5 and sAD = 5.8 ± 3 years). The cognitive impairment in MCI and AD subjects was determined by CDR and MMSE scores: CDR = 0 and MMSE = 24.8 ± 1.15 in MCI; CDR = 1-2 and MMSE = 19.1 ± 3 in mAD; CDR = 3 and MMSE = 16.29 ± 5.18 in sAD ($^{**}p < 0.001$ versus CTRL). All MCI subjects were amnestic, but 3 patients were single-domain amnestic and 7 patients multiple-domain amnestic MCI. The control group consisted of subjects who were diagnosed with no cognitive impairment based on MMSE scores (MMSE = 28.5 ± 2) and a clinical evaluation. Ten percent of the patients with MCI, 47.3% of those with mAD, and 11.7% of those with sAD were under cholinesterase inhibitor (ChEI) treatment. There were no differences in any other parameter evaluated between the patients receiving and those not receiving ChEI treatment (data not shown).

Plasma β-NGF concentration increased in MCI and mAD patients

ELISA showed that the mean plasma β-NGF concentration was significantly higher in the MCI and mAD patients, but not in the sAD patients (57.2 ± 11.6 pg/mL), than in the CTRL (133.1 ± 19.3 pg/mL and 105.6 ± 11.9 pg/mL, respectively, versus 51.8 ± 10.6 pg/mL; $^{**}p < 0.001$, $^{*}p < 0.05$) (Fig. 1A). Linear regression analysis showed a positive correlation between plasma β-NGF concentration and MMSE scores for all three patient groups ($r = 0.4567$, $r^2 = 0.2086$; $^{**}p = 0.0014$) (Fig. 1B).

Table 1
Demographic and clinical characteristics of AD, MCI, and CTRL subjects

	CTRL (n = 13)	MCI (n = 10)	Mild AD (n = 19)	Severe AD (n = 17)
Age (years ± SD)	76 ± 4	73.8 ± 2.9	77.6 ± 6.5	85.62 ± 6.2**
Gender F/M	5/8	6/4	7/12	10/7
Education (y ± SD)	6.3 ± 2	8.6 ± 2.6	7.36 ± 4.5	5.8 ± 3
Diagnosis (y ± SD)	–	2 ± 1.7	2.9 ± 1.8	2.9 ± 1.8
MCI types	–	3 a-sd MCI 7 a-md MCI	–	–
MMSE (mean ± SD)	28.5 ± 2	24.8 ± 1.15	19.1 ± 3**	16.29 ± 5.18**
CDR	–	0	1-2	3
ChEI	–	1 (10%)	9 (47.3%)	2 (11.7%)

CTRL, healthy subjects; MCI, mild cognitive impairment; AD, Alzheimer's disease; a-sd MCI, single-domain amnestic MCI; a-md MCI, multiple-domain amnestic MCI; MMSE, Mini-Mental State Examination; CDR, Clinical Dementia Rating scale; ChEI, cholinesterase inhibitor. $^{**}p < 0.001$ versus CTRL.

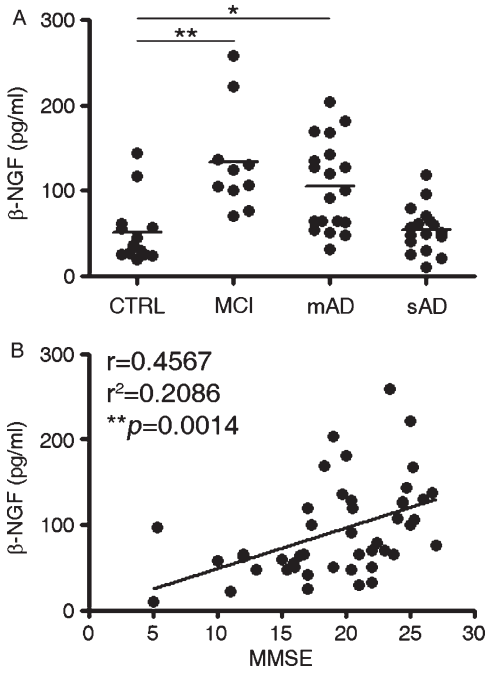

Fig. 1. Plasma β-NGF concentration increased in patients with MCI and mild AD. A) Plasma β-NGF levels in healthy subjects (CTRL) and in MCI, mAD (mild AD), and sAD (severe AD) patients. Horizontal bars indicate mean values. $^{**}p < 0.001$, $^{*}p < 0.05$. Comparisons between the groups were made using one-way ANOVA followed by Bonferroni multiple comparison tests. B) Correlation between plasma β-NGF concentration and MMSE scores in MCI, mAD, and sAD patients. r, Pearson index; r^2, coefficient of determination.

β-NGF receptor expression increased in monocytes isolated from MCI and AD patients

Using monocytes isolated from all subjects, we have monitored the surface expression of TrkA and p75NTR defined as percentage and mean fluorescent level (MFI).

The total percentage of TrKA positive monocytes did not change among any groups (CTRL = 87.4 ± 4.4%; MCI = 94.3 ± 1.7%; mAD = 91.2 ± 3.3%; sAD = 90.14 ± 5.2%) while, its MFI expression was significantly increased on monocytes isolated from MCI (180.7 ± 54.9) and mAD patients (131.7 ± 22.4) compared to CTRL subjects (56.08 ± 12.8, $^{*}p < 0.05$). No differences were found between sAD (84.3 ± 19.2) and CTRL monocytes (Fig. 2A).

The total percentage of p75NTR-positive monocytes and its MFI expression were significantly higher in the sAD patients (91.6 ± 1.3%; MFI 41.32 ± 3.3) as compared with the CTRL (67.8 ± 4.3%; MFI = 23.6 ± 2.8, $^{*}p < 0.05$), whereas no differences in either parameter were found between the MCI (76.0 ± 6.3%; MFI = 21.3 ± 3.7) and mAD patients (77 ± 3.5%; MFI = 22.5 ± 2.4) and the CTRL (Fig. 2B). Analysis of TrKA and p75NTR gene expression showed a significant increase in both genes for MCI patients (NTRK1 = 2.4 ± 0.18; NGFR = 1.9 ± 0.14) and mild AD (NTRK1 = 1.8 ± 0.05; NGFR = 2.5 ± 0.3) compared to CTRL (= 1, $^{*}p < 0.05$) (Fig. 2C, D).

Percentage of intermediate monocyte subset increased in MCI and mAD patients

There were no significant differences in the total monocyte percentage among the CTRL, the MCI, and the two AD groups (data not shown). Figure 3 shows the monocyte subsets based on the pattern of CD14 and CD16 expression. The percentage of classical monocytes, $CD14^{++}/CD16^{-}$, was significantly lower in MCI compared to CTRL (2.1 ± 0.3%, and 5.9 ± 0.57%, respectively, $^{*}p < 0.05$) while no statistically significant variation was found between both AD groups (mAD = 3.9 ± 0.8%; sAD = 4.6 ± 0.8%) and control cells as shown in Fig. 3A. Conversely, the percentage of intermediate monocytes, $CD14^{++}/CD16^{+}$, was significantly increased in MCI (4.8 ± 0.79%) and mAD (3.3 ± 0.4%) with respect to CTRL cells (1.8 ± 0.5, $^{*}p < 0.05$) while no differences were found between sAD (2.56 ± 0.37%) and control monocytes (Fig. 3B). No differences in the percentage of non-classical monocytes, $CD14^{+}/CD16^{++}$, were found among the four groups as shown in Fig. 3C (CTRL 0.47 ± 0.12; MCI 0.58 ± 0.16; mAD 0.54 ± 0.1; sAD 0.48 ± 0.11).

Analysis of surface expression of TrkA and p75NTR in relation to CD14 and CD16 expression on monocytes showed that TrKA expression in MCI was elevated in classical (30 ± 4.8) and intermediate/non classical (44 ± 3.6) subsets with respect to CTRL (11.3 ± 0.7 and 19.1 ± 1.9, respectively $^{*}p < 0.05$). Only intermediate/no classical subset of mAD showed a TrKA expression (29.8 ± 3.1) higher than CTRL ($^{*}p < 0.05$). Interestingly, in CTRL, MCI, and mAD, the expression of TrKA was significantly higher in intermediate/non classical subgroups with respect to the classical one ($^{*}p < 0.05$, Fig. 3D).

p75NTR expression was significantly higher in both monocyte subsets of sAD (classical = 36.44 ±

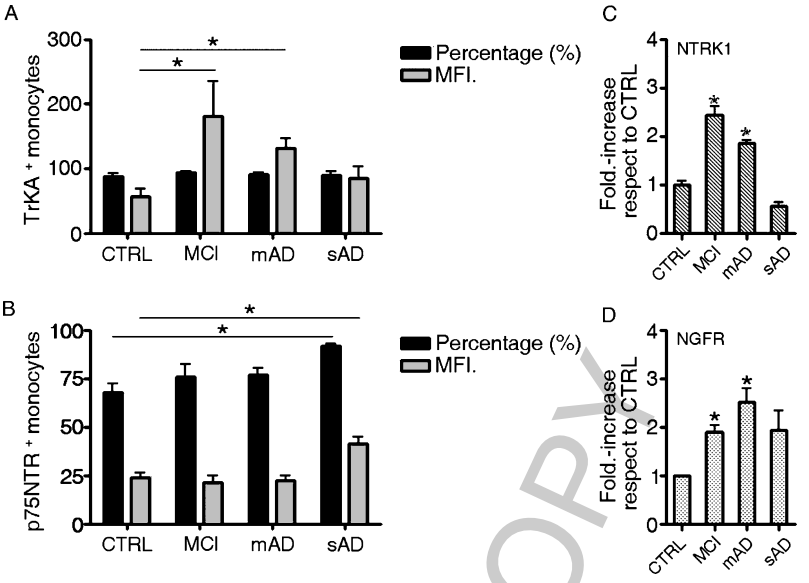

Fig. 2. Percentage of β-NGF receptors increased in monocytes from patients with MCI and mild AD. FACScan analysis was performed to measure the levels of TrKA (A) and p75NTR (B) expression on monocytes isolated from the peripheral blood of MCI, mAD, and sAD patients, and CTRL subjects. The results were expressed as percentage (grey bar) and mean fluorescence level (MFI, black bar). NTRK1 (C) and NGFR (D) gene expression was evaluated by qRT using a specific primer set (Material and Methods section). GAPDH was used as endogenous control. Data are presented as mean ± SD; $^{*}p < 0.05$; Comparisons between groups was performed by one-way ANOVA followed by Bonferroni multiple comparison tests.

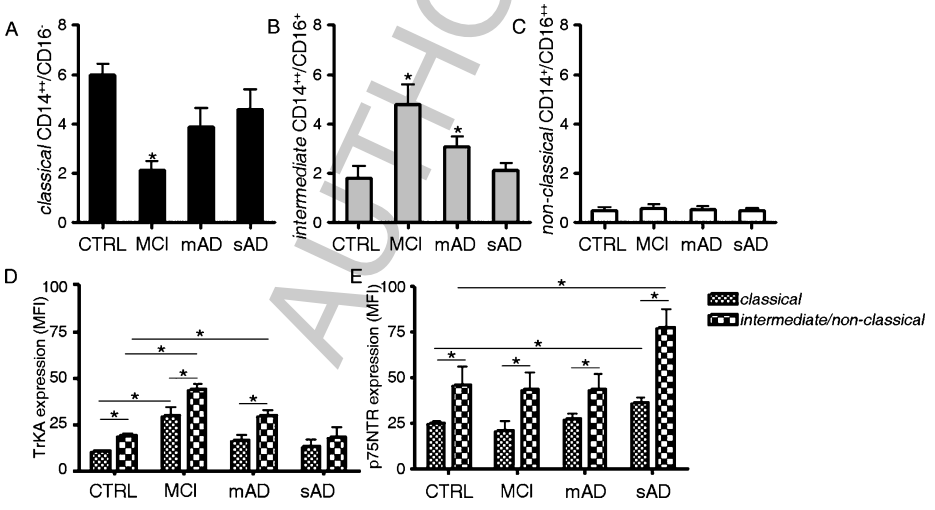

Fig. 3. Percentage of intermediate monocytes increased in patients with MCI and mild AD. FACScan analysis was performed to determine monocytes subsets based on patterns of CD14 and CD16 expression in blood isolated from patients with MCI, mAD, or sAD, and CTRL subjects. The presence of classical monocytes ($CD14^{++}/CD16^{-}$) (A), intermediate monocytes ($CD14^{++}/CD16^{+}$) (B), and non-classical monocytes ($CD14^{+}/CD16^{++}$) (C) was expressed as percentage of total mononuclear cells. Statistical analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison tests. The surface expression of TrKA (D) and p75NTR (E) was evaluated in association with CD14 (classical) and CD16 (intermediate/non-classical) expression on monocytes from all four groups. Results are presented as mean ± SD. $^{*}p < 0.05$. Student's *t*-test was performed to compare TrKA or p75NTR expression in classical and intermediate/non-classical monocytes; one-way ANOVA followed by Bonferroni multiple comparison tests were used for analysis between all groups.

3.2; intermediate/non classical = 77.13 ± 10.9) with respect to CTRL (classical = 25 ± 2.3; intermediate/non classical = 46.4 ± 9.6; $^{*}p < 0.05$) and also in this case the expression of p75NTR was highest in intermediate/non classical subgroups (Fig. 3E).

MCI and mAD showed a pro-inflammatory IL-6/IL-10 ratio

Intracellular levels of IL-6 and IL-10 cytokines were evaluated in unstimulated and Aβ-stimulated monocytes. IL-6-producing monocytes were increased in all groups after Aβ-stimulation with respect to unstimulated cells ($^{*}p < 0.05$) (Fig. 4A), confirming the pro-inflammatory stimulus of Aβ. IL-10-producing monocytes were significantly increased only in CTRL upon Aβ stimulation (12.4 ± 1.1 versus unstimulated, $^{*}p < 0.05$) and this value was higher than that observed in the stimulated cells from either the MCI (3.3 ± 0.7, $^{*}p < 0.05$) or the mAD patients (4.9 ± 1.3, $^{*}p < 0.05$) (Fig. 4B). The pro-inflammatory value, calculated as the ratio of IL-6- to IL-10-producing monocytes upon Aβ-stimulation, was higher in MCI (3.1 ± 0.3) and mAD (2.5 ± 0.55) with respect to CTRL (0.57 ± 0.17, $^{**}p < 0.001$ and $^{*}p < 0.05$) while no differences were found in sAD (1.45 ± 0.8) confirming the pro-inflammatory status of MCI and mAD subjects (Fig. 4C).

β-NGF and its receptors are linked to Aβ inflammatory-induced phenotype

As shown in Fig. 5A, *in vitro* addition of Aβ induced a significant increase in the release of β-NGF as compared with untreated monocytes (8.7 ± 0.6 pg/10^5 cells versus 1.6 ± 1.3 pg/10^5 cells, $^{**}p < 0.001$). As revealed by FACScan analysis, Aβ and β-NGF, either alone or together, consistently induced an increase in TrKA and p75NTR expression in with respect to the untreated cells ($^{*}p < 0.05$ versus UT), but the highest values for both receptors were obtained after β-NGF plus Aβ treatment, as shown in Fig. 5B (TrKA 345 ± 76 versus 133 ± 13.9; p75NTR 170.8 ± 10 versus 63.2 ± 4.2, $^{*}p < 0.05$ versus Aβ treatment). Evaluation of CD14 and CD16 expression on monocytes after treatment showed that β-NGF and Aβ, either alone or together, induced a reduction in classical monocytes, along with an increase in intermediate/non-classical monocytes as shown in Fig. 5C. Interestingly, IL-6 expression was increased in all treatments in a similar fashion with respect to untreated cells, while the intracellular level of IL-10 was significantly increased only in the cells treated with Aβ (Aβ = 327 ± 48, β-NGF = 171 ± 24, β-NGF+Aβ = 158 ± 11, $^{*}p < 0.05$ versus UT, β-NGF, and β-NGF+Aβ) (Fig. 5D). The ratio of IL-6- to IL-10-producing monocytes was higher in the monocytes treated with β-NGF (2.3 ± 0.2) and β-NGF plus Aβ (2.5 ± 0.3) than in

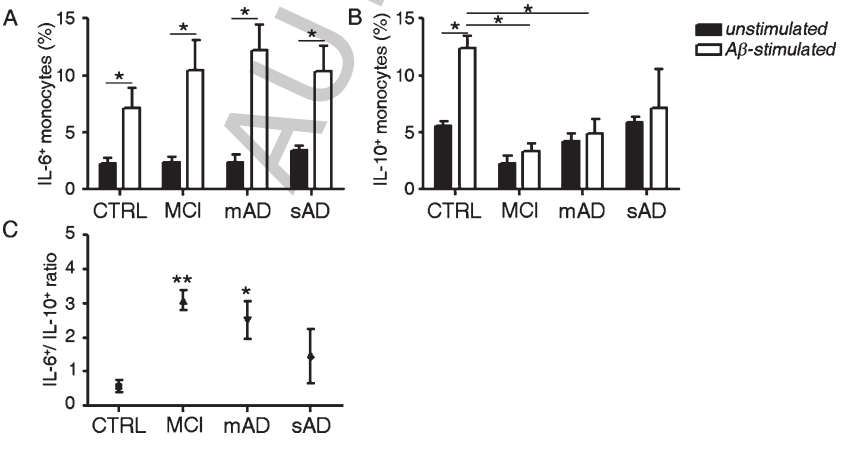

Fig. 4. Pro-inflammatory IL6 to IL10 ratio in patients with MCI and mild AD. Intracellular levels of IL6 (A) and IL10 (B) were evaluated by FACScan analysis in unstimulated (black bar) and Aβ-stimulated monocytes (white bar) isolated from MCI, mAD, and sAD patients and CTRL subjects. The pro-inflammatory value was calculated as the ratio of IL-6- to IL-10-producing monocytes upon Aβ stimulation (C). Data are presented as mean ± SD; $^{*}p < 0.05$. Student's *t*-test was used to compare the results of FACScan analysis between unstimulated and Aβ-stimulated monocytes; one-way ANOVA followed by Bonferroni multiple comparison tests were used for analysis between all groups.

the UT or those treated with Aβ alone (1.2 ± 0.7, $^{*}p < 0.05$) (Fig. 5E).

Percentage of monocytes in sub-G1 phase increased in sAD patients

Table 2 presents the sub-G1 phase distribution of monocytes with or without Aβ stimulation. The percentage of cells in sub-G1 phase was higher in the stimulated monocytes from the CTRL, MCI and mAD patients than in the unstimulated cells (CTRL $2.5 \pm 0.5\%$ versus 0.75 ± 0.25; MCI $1.9 \pm 0.1\%$ versus $0.6 \pm 0.2\%$; mAD $3.9 \pm 0.9\%$ versus $0.75 \pm 0.25\%$, respectively $^{*}p < 0.05$). Instead, the percentage of cells in sub-G1was higher in the unstimulated monocytes from the sAD patients than in those from the CTRL ($2.6 \pm 0.7\%$ versus 0.75 ± 0.25, $^{\S}p < 0.05$) and there was no significant increase after Aβ stimulation ($5.5 \pm 2.5\%$).

To verify activation of apoptosis, we determined the presence of cleaved caspase 3 by ELISA. We observed an increase in the percentage of monocytes in sub-G1 phase after Aβ stimulation in monocytes from CTRL, MCI, and mAD patients, along with an increase in intracellular cleaved caspase-3 levels with respect to unstimulated cells (0.3 ± 0.06 versus 0.12 ± 0.05; 0.4 ± 0.1 versus 0.14 ± 0.06; 0.3 ± 0.12 versus 0.18 ± 0.05 $^{*}p < 0.05$). Similar to the increase in sub-G1 percentage, cleaved caspase-3 was increased in the unstimulated monocytes from the sAD patients with respect to the unstimulated cells from the CTRL (0.4 ± 0.17 versus 0.12 ± 0.05, $^{\S}p < 0.05$) and no significant difference was observed upon Aβ stimulation ($0.85 \pm 0.1\%$).

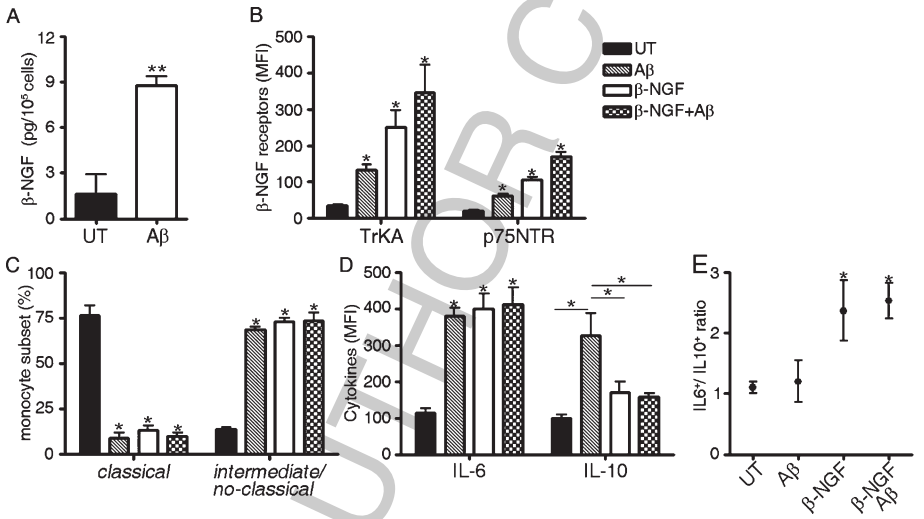

Fig. 5. β-NGF, TrKA, and p75NTR are linked to Aβ inflammatory-induced phenotype. Fresh monocytes isolated from CTRL subjects were incubated with Aβ, β-NGF alone or together (β-NGF+Aβ) as described in Material and Methods section. Results were compared with untreated cells (UT). After 24 h of treatment, we evaluated (A) concentration of β-NGF culture medium of Aβ−stimulated monocytes (Student's *t*-test); (B) TrKA and p75NTR monocytes expression reported as MFI by FACScan analysis; (C) Classical and intermediate/non-classical monocyte subsets based on CD14 and CD16 expression patterns; (D) Intracellular levels of IL-6 and IL-10; and (E) the pro-inflammatory value calculated as the ratio of IL-6- to IL-10-producing monocytes. Reported data are from five independent experiments and are expressed as mean ± SD. $^{*}p < 0.05$. Comparisons among all groups were performed by one-way ANOVA followed by Bonferroni multiple comparison tests.

Table 2
Cell cycle sub-G1 phase distribution of monocytes from healthy controls (CTRL) and from patients with MCI, mild AD (mAD), or severe AD (sAD) after stimulation with $A\beta_{42}$ peptide and presence of active caspase-3 (active-Casp3)

	CTRL		MCI		mAD		sAD	
	$-A\beta_{42}$	$+A\beta_{42}$	$-A\beta_{42}$	$+A\beta_{42}$	$-A\beta_{42}$	$+A\beta_{42}$	$-A\beta_{42}$	$+A\beta_{42}$
sub-G1 (%)	0.75 ± 0.25	$^{*}2.5 \pm 0.5$	0.6 ± 0.2	$^{*}1.9 \pm 0.1$	0.75 ± 0.25	$^{*}3.9 \pm 3.1$	$^{\S}2.6 \pm 0.7$	5.5 ± 2.5
active-Casp-3 (620 nm)	0.12 ± 0.05	$^{*}0.3 \pm 0.06$	0.14 ± 0.06	$^{*}0.4 \pm 0.1$	0.18 ± 0.05	$^{*}0.3 \pm 0.12$	$^{\S}0.4 \pm 0.17$	0.85 ± 0.1

$^{*}p < 0.05$ versus $-A\beta_{42}$. $^{\S}p < 0.05$ versus CTRL $-A\beta_{42}$.

DISCUSSION

The role of neurotrophins and their receptors in the CNS has been extensively studied in AD and other dementias, but little is known about their involvement in circulating blood cells during the progression of these degenerative diseases. For the first time, our study reports: i) an increase in plasma β-NGF concentration only during the early phases of dementia; ii) a variable modulation of TrKA and p75NTR expression on monocytes with the progression of dementia; iii) a positive association between TrKA expression and inflammatory status; iv) a positive association between p75NTR expression and caspase-3-mediated apoptosis.

A member of the cytokine superfamily, β-NGF is a key mediator of inflammation and a potent immune modulator. Monocytes and macrophages both synthesize β-NGF and express TrKA and p75NTR receptors [18, 19, 44]. In these cells, the binding of β-NGF to its receptors induces cell proliferation, survival/apoptosis, and regulates pro-inflammatory activities [45]. Furthermore, TrKA expression decreases during monocyte differentiation into macrophages [46]. On the contrary, little is known about the biological effects of the binding between β-NGF and p75NTR in monocytes and macrophages. We observed an increase in plasma β-NGF and higher MMSE scores in MCI and mAD subjects (early stage of dementia) compared to sAD subjects (late-stage dementia). With progression of the disease, MMSE scores decreased and plasma β-NGF similarly decreased in sAD patients and returned to control levels, suggesting that circulating β-NGF is involved during the initial stages of the disease. This observation is in agreement with Laske et al. [47] who demonstrated a similar peripheral trend for the serum concentration of brain-derived neurotrophic factor (BDNF). Because our data on plasma β-NGF concentration may include both the mature and the proNGF forms, future studies are needed to determine differences in the concentration of the two forms in MCI/mAD versus sAD.

Beside these changes in plasma β-NGF levels, the expression of TrkA and p75NTR on monocytes also changed with progression of dementia. We noted an increase in the MFI of TrKA-positive monocytes from MCI and mAD patients and an increase in both the percentage and the MFI of p75NTR-positive monocytes from sAD patients. These observations suggest that β-NGF and TrKA are involved only in the earliest phases of dementia followed by an increase in p75NTR in later phases.

Consistent with previous findings of a pro-inflammatory role for mono-macrophage lineage cells in AD [48–50], we also found a shift in the phenotype of circulating monocytes. In particular, we noted an increase in the inflammatory intermediate monocyte subset from the MCI and mAD patients, associated with a reduction in the non-inflammatory classical subset only in the MCI patients, whereas the sAD patients exhibited phenotypic patterns similar to the controls.

Though TrKA and p75NTR receptors were always expressed in both inflammatory and non-inflammatory subsets, their MFI values were higher in the inflammatory subsets. In particular, the MFI of TrKA was increased mainly in the MCI and mAD patients, whereas the MFI of p75NTR was increased mainly in the sAD patients. Interestingly, the increase in MFI of TrKA in the MCI and mAD patients was concomitant with the increase in plasma β-NGF concentration. This finding is line with similar phenomena we have found in other non-nervous-system-inflammatory diseases such as chronic obstructive pulmonary disease [17].

High levels of β-NGF and TrKA in the early stage of dementia promote inflammation, as demonstrated by a recent study that described their role in regulating the human innate immune response. In monocytic cell lines, the interaction between NGF and TrkA activates the inflammasome complex and induces the release of inflammatory cytokine IL-1β [51]. These results indicate that β-NGF receptors play a role in peripheral inflammatory status and their differential modulation with progression of dementia, with involvement of TrKA in the early stages and of p75NTR in the later phases.

Having noted these changes in monocytes during the progression of dementia, we then evaluated *in vitro* their cytokine production after Aβ stimulation [52, 53]. According to Sarasella et al. [48], we found that, with respect to unstimulated monocytes, IL-6 production was increased in the Aβ-stimulated monocytes from all subjects, whereas IL-10 production was increased only in the Aβ-stimulated monocytes from the healthy donors. In brief, stimulation with Aβ created a pro-inflammatory balance, defined as an IL-6-positive to IL-10-positive-monocyte ratio, in the patients with MCI and mAD.

To understand the reciprocal interactions between Aβ peptide, β-NGF, its receptors and monocyte

phenotypes, we treated the monocytes from control donors with β-NGF or Aβ alone or together. Our data indicate that stimulation with Aβ alone induces an increase in β-NGF production, while the ratio of IL-6 to IL-10 monocytes remains unchanged, whereas stimulation with β-NGF alone or Aβ plus β-NGF leads to a proinflammatory balance in the cytokine ratio. Furthermore, stimulation with Aβ plus β-NGF also resulted in the highest MFI value for TrKA and p75NTR on monocytes, indicating that a synergic effect exists between β-NGF and Aβ stimulation.

Since p75NTR expression is related to "cell death" [21], we analyzed cell cycle sub-G1 fraction and cleaved caspase-3 activation in order to understand the biological effects following the increase in p75NTR receptor expression on sAD monocytes. We found an increase in the percentage of sub-G1 fraction and cleaved caspase-3 after Aβ stimulation of monocytes from the sAD patients. These observations are shared by Bergman et al. [54] who reported a higher percentage of apoptotic PBMCs in AD patients with respect to healthy subjects.

Our results provide evidence for the hypothesis that, during MCI and the initial phases of AD, peripheral monocytes acquire an inflammatory phenotype in which β-NGF production and TrKA expression are increased. With progression of the disease, circulating Aβ and β-NGF accumulate and induce monocytes to increase p75NTR expression. Since in CNS the p75NTR binding of Aβ and its internalization is recognized as an attempt to degrade the peptide in lysosomes [25], it is reasonable to hypothesize that the increase in p75NTR expression on monocytes could have a similar function also for circulating Aβ peptides. However, since the ability of monocytes from AD patients to degrade Aβ peptides is constitutively impaired [11], the "scavenger" role of p75NTR in AD may be superseded by its classical role as death receptor with the consequent activation of cleaved caspase-3 [55] which, in turn, may be responsible for the increase in monocytic apoptosis described in AD [56].

Altogether, our results contribute to our understanding of the peripheral involvement of β-NGF and TrkA/p75NTR receptors in monocytes during in the progression of AD.

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REFERENCES

[1] Turner RS (2006) Alzheimer's disease. *Semin Neurol* **26**, 499-506.

[2] Heppner FL, Ransohoff RM, Becher B (2015) Immune attack: The role of inflammation in Alzheimer disease. *Nat Rev Neurosci* **16**, 358-372.

[3] Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, Axel L, Rusinek H, Nicholson C, Zlokovic BV, Frangione B, Blennow K, Ménard J, Zetterberg H, Wisniewski T, de Leon MJ (2015) Clearance systems in the brain-implications for Alzheimer disease. *Nat Rev Neurol* **11**, 457-470.

[4] Wang WY, Tan MS, Yu JT, Tan L (2015) Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Ann Transl Med* **3**, 136.

[5] Agostinho P, Cunha RA, Oliveira C (2010) Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Curr Pharm Des* **16**, 2766-2778.

[6] Feng Y, Li L, Sun XH (2011) Monocytes and Alzheimer's disease. *Neurosci Bull* **27**, 115-122.

[7] Malm TM, Koistinaho M, Pärepalo M, Vatanen T, Ooka A, Karlsson S, Koistinaho J (2005) Bone-marrow-derived cells contribute to the recruitment of microglial cells in response to beta-amyloid deposition in APP/PS1 double transgenic Alzheimer mice. *Neurobiol Dis* **18**, 134-142.

[8] Malm T, Koistinaho M, Muona A, Magga J, Koistinaho J (2010) The role and therapeutic potential of monocytic cells in Alzheimer's disease. *Glia* **58**, 889-900.

[9] Fiala M, Lin J, Ringman J, Kermani-Arab V, Tsao G, Patel A, Lossinsky AS, Graves MC, Gustavson A, Sayre J, Sofroni E, Suarez T, Chiappelli F, Bernard G (2005) Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *J Alzheimers Dis* **7**, 221-232.

[10] Fiala M, Cribbs DH, Rosenthal M, Bernard G (2007) Phagocytosis of amyloid-beta and inflammation: Two faces of innate immunity in Alzheimer's disease. *J Alzheimers Dis* **11**, 457-463.

[11] Tiribuzi R, Crispoltoni L, Porcellati S, Di Lullo M, Florenzano F, Pirro M, Bagaglia F, Kawarai T, Zampolini M, Orlacchio A, Orlacchio A (2014) miR128 up-regulation correlates with impaired amyloid β(1-42) degradation in monocytes from patients with sporadic Alzheimer's disease. *Neurobiol Aging* **35**, 345-356.

[12] Guerreiro RJ, Santana I, Brás JM, Santiago B, Paiva A, Oliveira C (2007) Peripheral inflammatory cytokines

as biomarkers in Alzheimer's disease and mild cognitive impairment. *Neurodegener Dis* **4**, 406-412.

[13] Hochstrasser T, Marksteiner J,Humpel C (2012) Telomere length is age-dependent and reduced in monocytes of Alzheimer patients. *Exp Gerontol* **47**, 160-163.

[14] Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC (2012) The three human monocyte subsets: Implications for health and disease. *Immunol Res* **53**, 41-57.

[15] Pistilli A, Rende M, Crispoltoni L, Montagnoli C, Stabile AM (2015) LY294002 induces in vitro apoptosis and overexpression of p75NTR in human uterine leiomyosarcoma HTB 114 cells. *Growth Factors* **33**, 376-383.

[16] Wang W, Chen J, Guo X (2014) The role of nerve growth factor and its receptors in tumorigenesis and cancer pain. *Biosci Trends* **8**, 68-74.

[17] Stabile A, Pistilli A, Crispoltoni L, Montagnoli C, Tiribuzi R, Casali L, Rende M (2016) A role for NGF and its receptors TrKA and p75NTR in the progression of COPD. *Biol Chem* **397**, 157-163.

[18] Freund-Michel V, Frossard N (2008) The nerve growth factor and its receptors in airway inflammatory diseases. *Pharmacol Ther* **117**, 52-76.

[19] Otten U, Scully JL, Ehrhard PB, Gadient RA (1994) Neurotrophins: Signals between the nervous and immune systems. *Prog Brain Res* **103**, 293-305.

[20] Chao MV (2003) Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nat Rev Neurosci* **4**, 299-309.

[21] Meeker R, Williams K (2014) Dynamic nature of the p75 neurotrophin receptor in response to injury and disease. *J Neuroimmune Pharmacol* **9**, 615-628.

[22] Lambiase A, Micera A, Sgrulletta R, Bonini S, Bonini S (2004) Nerve growth factor and the immune system: Old and new concepts in the cross-talk between immune and resident cells during pathophysiological conditions. *Curr Opin Allergy Clin Immunol* **4**, 425-430.

[23] Iulita MF, Cuello AC (2016) The NGF metabolic pathway in the CNS and its dysregulation in down syndrome and Alzheimer's disease. *Curr Alzheimer Res* **13**, 53-67.

[24] Costantini C, Scrable H, Puglielli L (2006) An aging pathway controls the TrkA to p75NTR receptor switch and amyloid beta-peptide generation. *EMBO J* **25**, 1997-2006.

[25] Ovsepian SV, Antyborzec I, O'Leary VB, Zaborszky L, Herms J, Oliver Dolly J (2014) Neurotrophin receptor p75 mediates the uptake of the amyloid beta (Aβ) peptide, guiding it to lysosomes for degradation in basal forebrain cholinergic neurons. *Brain Struct Funct* **219**, 1527-1541

[26] Hellweg R, Gericke CA, Jendroska K, Hartung HD, Cervós-Navarro J (1998) NGF content in the cerebral cortex of non-demented patients with amyloid-plaques and in symptomatic Alzheimer's disease. *Int J Dev Neurosci* **16**, 787-794.

[27] Hock C, Heese K, Hulette C, Rosenberg C, Otten U (2000) Region-specific neurotrophin imbalances in Alzheimer disease: Decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch Neurol* **57**, 846-851.

[28] Rojo LE, Fernández JA, Maccioni AA, Jimenez JM, Maccioni RB (2008) Neuroinflammation: Implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. *Arch Med Res* **39**, 1-16.

[29] Hock C, Heese K, Müller-Spahn F, Huber P, Riesen W, Nitsch RM, Otten U (2000) Increased CSF levels of nerve growth factor in patients with Alzheimer's disease. *Neurology* **54**, 2009-2011.

[30] Mashayekhi F, Salehin Z (2006) Cerebrospinal fluid nerve growth factor levels in patients with Alzheimer's disease. *Ann Saudi Med* **26**, 278-282.

[31] Blasko I, Lederer W, Oberbauer H, Walch T, Kemmler G, Hinterhuber H, Marksteiner J, Humpel C (2006) Measurement of thirteen biological markers in CSF of patients with Alzheimer's disease and other dementias. *Dement Geriatr Cogn Disord* **21**, 9-15.

[32] Counts SE, He B, Prout JG, Michalski B, Farotti L, Fahnestock M, Mufson EJ (2016) Cerebrospinal fluid proNGF: A putative biomarker for early Alzheimer's disease. *Curr Alzheimer Res* **13**, 800-808.

[33] Schaub RT, Anders D, Golz G, Göhringer K, Hellweg R (2002) Serum nerve growth factor concentration and its role in the preclinical stage of dementia. *Am J Psychiatry* **159**, 1227-1229.

[34] Faria MC, Gonçalves GS, Rocha NP, Moraes EN, Bicalho MA, Gualberto Cintra MT, Jardim de Paula J, José Ravic de Miranda LF, Clayton de Souza Ferreira A, Teixeira AL, Gomes KB, Carvalho MD, Sousa LP (2014) Increased plasma levels of BDNF and inflammatory markers in Alzheimer's disease. *J Psychiatr Res* **53**, 166-172.

[35] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939-944.

[36] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-Mental State". A practical method for grading the cognitive state of patients for the clinicians. *J Psychiatr Res* **12**, 189-198.

[37] Morris JC (1997) Clinical Dementia rating: A reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. *Int Psychogeriatr* **9**, 173-176.

[38] Chaves ML, Camozzato AL, Godinho C, Kochhann R, Schuh A, de Almeida VL, Kaye J (2007) Validity of the clinical dementia rating scale for the detection and staging of dementia in Brazilian patients. *Alzheimer Dis Assoc Disord* **21**, 210-217.

[39] Tiribuzi R, Orlacchio A, Crispoltoni L, Maiotti M, Zampolini M, De Angelis M, Mecocci P, Cecchetti R, Bernardi G, Datti A, Martino S, Orlacchio A (2011) Lysosomal β-galactosidase and β-hexosaminidase activities correlate with clinical stages of dementia associated with Alzheimer's disease and type 2 diabetes mellitus. *J Alzheimers Dis* **24**, 785-797.

[40] Abeles RD, McPhail MJ, Sowter D, Antoniades CG, Vergis N, Vijay GK, Xystrakis E, Khamri W, Shawcross DL, Ma Y, Wendon JA, Vergani D (2012) CD14, CD16 and HLA-DR reliably identifies human monocytes and their subsets in the context of pathologically reduced HLA-DR expression by $CD14^{hi}/CD16^{neg}$ monocytes: Expansion of $CD14^{hi}/CD16^{pos}$ and contraction of $CD14^{lo}/CD16^{pos}$ monocytes in acute liver failure. *Cytometry A* **81**, 823-834.

[41] Williams KS, Killebrew DA, Clary GP, Meeker RB (2016) Opposing effects of NGF and proNGF on HIV induced macrophage activation. *J Neuroimmune Pharmacol* **11**, 98-120.

[42] Foveau B, Ancot F, Leroy C, Petrelli A, Reiss K, Vingtdeux V, Giordano S, Fafeur V, Tulasne D (2009) Down-regulation of the met receptor tyrosine kinase by presenilin-dependent regulated intramembrane proteolysis. *Mol Biol Cell* **20**, 2495-2507.

[43] Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* **3**, 1101-1108.

[44] Hoyle GW (2003) Neurotrophins and lung disease. *Cytokine Growth Factor Rev* **14**, 551-558.

[45] Williams KS, Killebrew DA, Clary GP, Seawell JA, Meeker RB (2015) Differential regulation of macrophage phenotype by mature and pro-nerve growth factor. *J Neuroimmunol* **285**, 76-93.

[46] El Zein N, D'Hondt S, Sariban E (2010) Crosstalks between the receptors tyrosine kinase EGFR and TrkA and the GPCR, FPR, in human monocytes are essential for receptors-mediated cell activation. *Cell Signal* **22**, 1437-1447.

[47] Laske C, Stransky E, Leyhe T, Eschweiler GW, Wittorf A, Richartz E, Bartels M, Buchkremer G, Schott K (2006) Stage-dependent BDNF serum concentrations in Alzheimer's disease *J Neural Transm (Vienna)* **113**, 1217-1224.

[48] Saresella M, Marventano I, Calabrese E, Piancone F, Rainone V, Gatti A, Alberoni M, Nemni R, Clerici M (2014) A complex proinflammatory role for peripheral monocytes in Alzheimer's disease. *J Alzheimers Dis* **38**, 403-413.

[49] Thériault P, ElAli A, Rivest S (2015) The dynamics of monocytes and microglia in Alzheimer's disease. *Alzheimers Res Ther* **7**, 41.

[50] Ciaramella A, Bizzoni F, Salani F, Vanni D, Spalletta G, Sanarico N, Vendetti S, Caltagirone C, Bossú P (2010) Increased pro-inflammatory response by dendritic cells from patients with Alzheimer's disease. *J Alzheimers Dis* **19**, 559-572.

[51] Datta-Mitra A, Kundu-Raychaudhuri S, Mitra A, Raychaudhuri SP (2015) Cross talk between neuroregulatory molecule and monocyte: Nerve growth factor activates the inflammasome. *PLoS One* **10**, e0121626.

[52] Ferretti MT, Cuello AC (2011) Does a pro-inflammatory process precede Alzheimer's disease and mild cognitive impairment? *Curr Alzheimer Res* **8**, 164-174.

[53] Pellicanò M, Bulati M, Buffa S, Barbagallo M, Di Prima A, Misiano G, Picone P, Di Carlo M, Nuzzo D, Candore G, Vasto S, Lio D,Caruso C, Colonna-Romano G (2010) Systemic immune responses in Alzheimer's disease: In vitro mononuclear cell activation and cytokine production. *J Alzheimers Dis* **21**, 181-192.

[54] Bergman M, Salman H, Beloosesky Y, Djaldetti M, Bessler H (2002) Are peripheral blood cells from patients with Alzheimer disease more sensitive to apoptotic stimuli? *Alzheimer Dis Assoc Disord* **16**, 156-160.

[55] Zeng F, Lu JJ, Zhou XF, Wang YJ (2011) Roles of p75NTR in the pathogenesis of Alzheimer's disease: A novel therapeutic target. *Biochem Pharmacol* **821**, 1500-1509.

[56] Yi CH, Yuan J (2009) The Jekyll and Hyde functions of caspases. *Dev Cell* **16**, 21-34.