Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-191250
IOS Press

Protective Role of Cerebrospinal Fluid Inflammatory Cytokines in Patients with Amnestic Mild Cognitive Impairment and Early Alzheimer's Disease Carrying Apolipoprotein E4 Genotype

Caterina Motta[a,b,*], Annamaria Finardi[c], Sofia Toniolo[b], Francesco Di Lorenzo[b], Eugenia Scaricamazza[b], Stefano Loizzo[d], Nicola Biagio Mercuri[b], Roberto Furlan[c], Giacomo Koch[a] and Alessandro Martorana[b]

[a]*Non Invasive Brain Stimulation Unit/Department of Behavioral and Clinical Neurology, Santa Lucia Foundation IRCCS, Rome, Italy*
[b]*Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy*
[c]*Clinical Neuroimmunology Unit, Department of Neuroscience, Institute of Experimental Neurology (InSpe), San Raffaele Scientific Institute, Milan, Italy*
[d]*Center for Global Health, Italian National Institute of Health, Rome, Italy*

Accepted 4 May 2020

Abstract.
Background: Neuroinflammatory cytokines can play a pivotal role in Alzheimer's disease (AD) contributing to the evolution of degenerative processes.
Objective: We aimed at evaluating the levels of cerebrospinal fluid (CSF) inflammatory cytokines, chemokines, and growth factors in subjects with diagnosis of amnestic mild cognitive impairment and mild AD.
Methods: We evaluated CSF contents of inflammatory cytokines in 66 patients divided according to the NIA-AA research framework and the *APOE* genotype. CSF of a group of cognitively unimpaired individuals ($n = 23$) was evaluated as control. All patients were evaluated for 24 months using Mini-Mental State Examination (MMSE).
Results: We found significant increased levels of IL-4, IL-6, IL-8, and G-CSF in the CSF of A+/T– *APOE4* carriers, respect to A+/T– patients homozygous for *APOE3*, respect to A+/T+ patients, regardless the *APOE* status, and respect to controls. Over a period of 24 months, A+/T– *APOE4* carriers, with increased levels of cytokines, showed a preserved cognitive evaluation when compared to the other subgroups of patients (delta MMSE at 24 months respect to baseline: 0.10 ± 0.35; $p < 0.05$).
Conclusion: Our data suggest that during early phases of AD, in *APOE4* carriers, Aβ pathology likely induces a specific cytokines pattern synthesis associated to cognitive preservation. These data highlight the different role that neuroinflammation can play in AD pathology based on the presence of specific CSF biomarkers and on the *APOE* status.

Keywords: Amyloid-β 42, *APOE*, cognitive decline, G-CSF, interleukins, tau

Uncorrected Author Proof

*Correspondence to: Caterina Motta, MD, Non Invasive Brain Stimulation Unit/Department of Behavioral and Clinical Neurology, Santa Lucia Foundation IRCCS, Rome, Italy. Tel.: +39 0651501181; E-mail: c.motta@hsantalucia.it.

ISSN 1387-2877/20/$35.00 © 2020 – IOS Press and the authors. All rights reserved

INTRODUCTION

Alzheimer's disease (AD) is a multifactorial, chronic neurodegenerative disorder, which main pathological features are the extracellular senile plaques and the intraneuronal neurofibrillary tangles [1]. In last decades, neuropathological analysis of AD brains revealed that neuroinflammation is an important driving force for neurodegeneration and AD progression [2]. During physiological aging and in AD, cytokines levels increase and set neuronal environment in an inflammatory state [3], contributing to the evolution of degenerative process. Neuroinflammation is a complex mechanism mediated by cytokines mainly released by microglial cells and astrocytes, whose activation may have both detrimental or protective role for neurons. Beneficial pro-inflammatory cytokines are protective when involved in the induction and modulation of neuronal growth, cell survival, and modulation of synaptic plasticity mechanisms. Conversely, a prolonged and aberrant pro-inflammatory signaling is responsible for surrounding tissue neurodegeneration [4]. Microglial cells play a key role in the inflammatory process of the central nervous system (CNS) and represent a major focus of neurodegenerative disease research. Microglia could remain in balance between a pro-inflammatory status (M1 phenotype), characterized by the synthesis of inflammatory cytokines such as interleukin 1 β (IL-1β), IL-6, and tumor necrosis factor (TNF), counteracted by the synthesis and release of anti-inflammatory cytokines (IL-4, IL-8, and IL-10) and neurotrophic factors (M2 phenotype), depending on the specific stimulus the microglia has been exposed to [5]. Thus, in such intricate scenario, the complex role of inflammatory cytokines in both neurodegeneration and neuroprotection is far from completion. In AD amyloid-β (Aβ) peptides, including both the oligomeric and the senile plaques forms, are considered main trigger for inflammatory signaling [6]. In particular, a prolonged proinflammatory signaling due to Aβ mis-metabolism, can lead to overproduction of pro-inflammatory cytokines involved in the neurodegenerative pathways signaling [4]. On the other hand, there is evidence that an increased Aβ production arises as a direct result of prolonged neuroinflammation [7]. It is important to note that in AD most of the modulatory effects of cytokines are related to the amyloid cascade signaling [8], while cognitive dysfunction progression is rather related to neuronal degeneration and tau-pathology [9]. Tau protein, part of the neuronal cytoskeleton, is necessary for axonal physiology, for neurite outgrowth, neural plasticity mechanisms, repair of neurons after injuries [10], and even a regulatory role for cell firing has recently been described, giving to this protein a wider function than previously believed [11]. Impaired metabolism of tau protein has been demonstrated to rapidly induce impairment of neurotransmission and synaptic plasticity, all mechanisms responsible for cognitive decline in AD patients [12–15]. However, a clear relationship between tau pathology and neuroinflammation is still unclear. We recently showed that human astrocytes cultures incubated with cerebrospinal fluid (CSF) samples from AD patients were vulnerable in terms of increased apoptosis only in the presence of high levels of tau protein and *APOE4* genotype [16]. Such findings led us to hypothesize a major role for tau protein in astrocytes degeneration and likely a proinflammatory role for tau in *APOE4* individuals [17]. Interestingly, it has been reported that *APOE4* carriers present an unbalanced switching of the microglial phenotype M1–M2 [18]. Moreover, microglial ApoE downstream regulates the microglial homeostatic gene expression, leading to a neurodegenerative-associated phenotype switch, which could further promote AD pathology [19].

Thus, the main objective of this study was to evaluate the levels of 15 cytokines in the CSF of patients with amnestic mild cognitive impairment (aMCI) and mild AD, according to the hallmarks processes of amyloid deposition, tau pathology, and *APOE* genotype. To reduce possible discrepancies between clinical presentation and CSF biomarkers profile, patients were divided using the NIA-AA research framework [20]. The AT(N) classification divides biomarkers into amyloid deposits (A), neurofibrillary tangles (T), and neurodegeneration (N), determined by measuring CSF levels of $A\beta_{42}$, phosphorylated tau (p-tau), and total tau (t-tau), respectively. Although biomarkers of neurodegeneration (N) provide important pathologic staging information they are not specific for neurodegeneration due to AD; for this reason, A and T biomarkers are commonly used to discriminate patients in the AD continuum, subdivided into AD pathologic change (A+/T–) and AD (A+/T+). Because of the evidence of modulation of ApoE isoforms in neuroinflammation [17–19] and the effect of neuroinflammation on the neurodegenerative processes in several types of dementia [21], we expect to find a different profile of neuroinflammatory cytokines in patients classified by AT and

Uncorrected Author Proof

APOE genotype. Furthermore, we expect to find different rate of disease progression among groups, thus patients were evaluated with neuropsychological testing for a period of two years.

METHODS

Subjects

Sixty-six consecutive patients (range, 58–79 years; median, 71) were recruited at the memory clinic of the University Hospital Tor Vergata, admitted for complaining memory symptoms. The diagnosis of probable or possible AD fulfilled the criteria of the National Institute on Aging and Alzheimer's Association (NIA/AA) [22] and all patients had a mild disease with Mini-Mental State Examination (MMSE) scores ranging 20–24. The aMCI patients were diagnosed using the NIA/AA criteria for MCI [23]. All patients underwent, for diagnostic purposes, a complete clinical investigation in a period not superior to 60 days, including medical history, neurological examination, MMSE, a complete blood screening, and neuropsychological assessment [24] including the following cognitive domains: general cognitive efficiency: MMSE; verbal episodic memory: Rey auditory verbal long-term memory (15-Word List Immediate and 15 min Delayed recall); visuospatial abilities and visuospatial episodic memory: Complex Rey's Figure (copy and 10 min Delayed recall); and executive functions: phonological word fluency; analogic reasoning: Raven's Colored Progressive Matrices. Patients underwent also a neuropsychiatric evaluation, magnetic resonance or computed tomography (CT) imaging, positron emission tomography/CT, and lumbar puncture for CSF analysis. Exclusion criteria were: cognitive isolated deficits, clinically manifest acute stroke in the last 6 months showing a Hachinski scale score >4, and a radiological evidence of ischemic lesions, $A\beta_{1-42}$ CSF values >600 pg/mL. All patients started treatment with rivastigmine patch or donepezil and were followed longitudinally with clinical assessments and MMSE testing at 6, 12, and 18 months.

Control patients ($n = 23$) were evaluated for headache in the Policlinico Tor Vergata Hospital Emergency Department between October 2014 and December 2015, and the CSF samples were collected in accordance with standard hospital practice. The control subjects did not carry a diagnosis of active infection and were free of cognitive and primary neurological disorders other than headache.

All participants or their legal guardian provided written informed consent after receiving an extensive description of the study. The study was performed according to the Declaration of Helsinki. The ethics committee of the Santa Lucia Foundation approved this protocol (Prot. CE/AG4/PROG.392-08).

Biomarkers collection and genotype analysis

The first 12 mL of CSF were collected in a polypropylene tube and directly transported to the local laboratory for centrifugation at 2000 g at +4°C for 10 min. The supernatant was pipetted off, mixed to avoid potential gradient effects and aliquoted in 1 mL portions in polypropylene tubes, stored at –80°C pending biochemical analyses. CSF t-tau and p-tau phosphorylated at Thr181 concentrations were determined using a sandwich enzyme-linked immunosorbent assay (ELISA; Innotest hTAU-Ag; Innogenetics, Gent, Belgium). $A\beta_{1-42}$ levels were determined using a sandwich ELISA (Innotest β-amyloid; Innogenetics) [25]. Genotyping for *APOE* were performed by allelic discrimination technology (TaqMan; Applied Biosystems).

CSF cytokines and chemokines determination

In a group of 89 individuals CSF contents of cytokines and chemokines were determined. These include IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, tumor necrosis factor-alpha (TNFα); granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF); macrophage inflammatory proteins (MIP)-1a and monocyte chemotactic protein 1 (MCP-1). To determine cytokines and chemokines, the CSF was centrifuged and immediately stored at –80°C until analyzed using Bio-Plex Multiplex Cytokine Assay (Bio-Rad Laboratories, Hercules, CA), according to manufacturer's instructions. Concentration of analytes were calculated according to a standard curve and expressed as picograms per milliliter. When the concentrations of the analytes were below the detection threshold, they were assumed to be 0 pg/ml; a maximum of values below the limit of detection of 5% for each cytokine was considered acceptable for the analysis.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Differences among groups were compared

Uncorrected Author Proof

Table 1
Demographical and clinical data of healthy controls and patients divided using the NIA-AA classification and *APOE* genotype

	HC ($n=23$)	(A+/T–) E3 ($n=20$)	(A+/T–) E4 ($n=16$)	(A+/T+) E3 ($n=19$)	(A+/T+) E4 ($n=11$)	p
Age, y (mean ± SD)	66.8 ± 7.6	70.3 ± 6.4	70.7 ± 5.6	69.8 ± 7.1	70.0 ± 6.3	0.34
Female (%)	59%	60%	62%	63%	63%	0.89
Disease duration, m (mean ± SD)	n.a.	11.3 ± 3.5	10.9 ± 3.3	11.3 ± 3.3	11.2 ± 3.9	0.99
Education, y (mean ± SD)	n.a.	8.4 ± 3.6	9.0 ± 3.5	9.1 ± 3.9	9.1 ± 3.5	0.93
MMSE (mean ± SD)	n.a.	24.6 ± 2.1	24.1 ± 1.8	24.2 ± 2.1	23.9 ± 2.1	0.99
CSF total-tau, pg/ml (mean ± SD)	n.a.	244.5 ± 112.1	267.1 ± 90.1	720.1 ± 217.8	707.4 ± 220	<0.01
CSF p-tau, pg/ml (mean ± SD)	n.a.	41.1 ± 15.9	47.2 ± 16.1	89.4 ± 23.4	86.3 ± 27.9	<0.01
CSF Abeta 1–42, pg/ml (mean ± SD)	n.a.	356.6 ± 97.2	377.7 ± 124.6	367.7 ± 99.2	399.6 ± 94.7	0.73
Diabetes (%)	13.1	20.0	18.7	21.1	18.2	0.97
Hypertension (%)	34.8	30.0	31.3	31.6	36.3	0.99
Hyperlipidemia (%)	30.4	30.0	31.3	31.6	27.3	0.98
Arthritis (%)	4.3	5.0	12.5	15.8	9.1	0.68
Thyroiditis (%)	8.7	15.0	12.5	15.8	18.2	0.93
COPD (%)	8.7	10.0	6.3	10.5	9.1	0.99
Cancer (%)	8.7	5.0	0	0	0	0.42
Multimorbidity (%)	26.1	20.0	31.2	31.6	27.3	0.92

n, numbers; y, years; m, months; SD, standard deviation; MMSE, Mini-Mental State Examination; CSF, cerebrospinal fluid; COPD, chronic obstructive pulmonary disease; n.a., not applicable.

by univariate analysis using one-way ANOVA for continuous variables and Fisher Exact Test for categorical variables. The Tuckey test was used for *post hoc* multiple comparison. All statistical analyses were conducted using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). A *p*-value (p) of less than 0.05 was considered statistically significant.

RESULTS

Sixty-six consecutive patients were recruited at the memory clinic of the University Hospital Tor Vergata. All patients showed neuropsychological profile compatible with a diagnosis of aMCI or mild AD. Based on AT/N classification patients were grouped in (A+/T–) E4, (A+/T–) E3, (A+/T+) E4, and (A+/T+) E3. Groups did not differ in gender, education, age at disease onset, disease duration, MMSE score at baseline, chronic medical conditions, and multimorbidity (defined as the coexistence of two or more chronic conditions in the same individual) as shown in Table 1. Twenty-three cognitively unimpaired subjects, evaluated for headache in the Policlinico Tor Vergata Hospital Emergency Department, underwent CSF sampling in accordance with standard hospital practice and were used as control subjects.

CSF levels of cytokines, chemokines, and growth factors according to NIA-AA research framework and APOE genotype

In this experimental setting cytokines, chemokines and growth factor's levels (IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, TNFα; G-CSF, GM-CSF; MIP-1 and MCP-1) were determined in CSF samples of each group of patients and controls. Results showed that differences among groups reached statistical significance only for G-CSF ($F=6.463$; $p<0.001$), IL-4 ($F=4.059$; $p=0.004$), IL-6 ($F=4.481$; $p=002$), and IL-8 ($F=5.296$; $p<0.001$) (see Table 2). In particular, in the multiple comparisons analyses, we found that G-CSF and IL-4 levels were significantly higher in the (A+/T–) E4 group ($p<0.05$ for all comparisons) (Fig. 1A, B). Similarly, we found significant higher levels of IL-6 and IL-8 in the CSF of (A+/T–) E4 group respect to (A+/T–) E3 group, (A+/T+) E4 group, and controls ($p<0.05$ for all comparisons), as well as a strong tendency compared to the (A+/T+) E3 group ($p=0.06$ for IL-6, $p=0.09$ for IL-8) (Fig. 1C, D).

Cognitive decline over 24 months

Patients were then clinically followed over a period of 24 months in our memory clinic. Results showed a significant difference after 12 months in clinical progression (evaluated as delta MMSE scores respect

Uncorrected Author Proof

Table 2
CSF levels of cytokines and chemokines in healthy controls and patients divided by *APOE* genotype

	HC	(A+/T–) E3	(A+/T–) E4	(A+/T+) E3	(A+/T+) E4	*p*
IL-1beta	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.04	0.09 ± 0.02	0.10 ± 0.05	0.697
IL-2	0.70 ± 0.09	0.61 ± 0.13	0.50 ± 0.17	0.56 ± 0.12	0.57 ± 0.13	0.898
IL-4	0.19 ± 0.02	0.21 ± 0.03	0.37 ± 0.06	0.21 ± 0.03	0.17 ± 0.02	0.004
IL-6	3.51 ± 0.33	4.69 ± 0.83	9.71 ± 2.08	5.32 ± 1.02	4.54 ± 0.97	0.002
IL-7	7.81 ± 0.98	9.70 ± 1.28	11.54 ± 1.94	6.90 ± 1.48	6.96 ± 2.31	0.175
IL-8	20.68 ± 0.85	20.82 ± 1.70	30.11 ± 3.27	23.86 ± 0.97	19.74 ± 1.29	<0.001
IL-10	2.87 ± 0.14	2.81 ± 0.07	2.78 ± 0.14	2.90 ± 0.12	3.07 ± 0.31	0.425
IL-12	1.21 ± 0.23	1.21 ± 0.25	1.76 ± 0.23	1.39 ± 0.25	1.26 ± 0.25	0.476
IL-13	1.99 ± 0.52	1.57 ± 0.26	1.63 ± 0.45	1.46 ± 0.27	1.92 ± 0.56	0.845
IL-17	1.96 ± 0.39	1.65 ± 0.38	1.88 ± 0.36	2.20 ± 0.39	2.07 ± 0.51	0.893
G-CSF	4.14 ± 0.45	5.74 ± 0.77	9.22 ± 1.21	5.011 ± 0.57	4.88 ± 0.83	<0.001
GM-CSF	40.77 ± 5.28	50.49 ± 5.96	51.99 ± 7.00	57.55 ± 5.94	46.48 ± 8.53	0.364
MCP-1	349.9 ± 25.8	320.1 ± 18.2	330.3 ± 27.2	311.5 ± 19.7	322.7 ± 22.7	0.447
MIP-1b	10.15 ± 0.58	9.88 ± 0.64	12.35 ± 1.68	12.55 ± 1.15	12.43 ± 1.02	0.152
TNFα	1.84 ± 0.21	1.52 ± 0.22	1.85 ± 0.39	1.93 ± 0.25	1.86 ± 0.24	0.800

HC, healthy controls; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein 1; MIP-1b, macrophage inflammatory proteins 1b; TNFα, tumor necrosis factor-α.

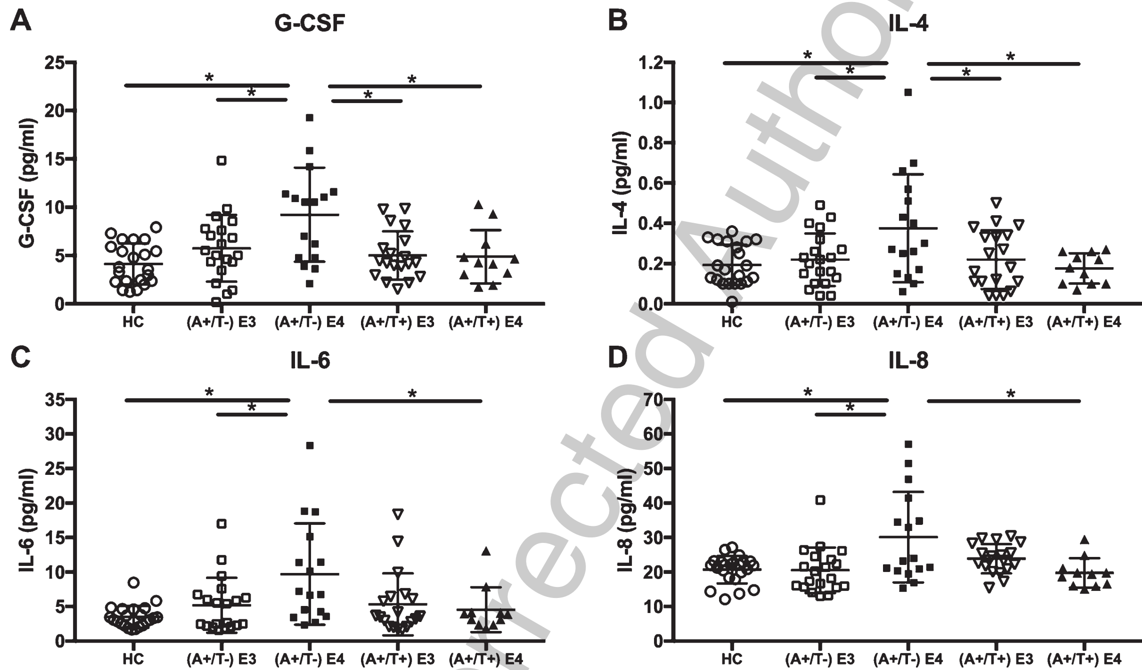

Fig. 1. Multiple comparisons of G-CSF (A), IL-4 (B), IL-6 (C), and IL-8 (D) CSF levels among controls and patients' groups according to *APOE* genotype. $^{*}p < 0.05$.

to baseline) for (A+/T–) E4 patients (0.86 ± 0.44), with respect to (A+/T+) E3 (–1.41 ± 0.61; $p = 0.048$) and to (A+/T+) E4 (–2.70 ± 0.90; $p = 0.005$), but not to (A+/T–) E3 patients (–0.72 ± 0.64; $p = 0.276$) (Fig. 2A). Similarly, at 24 months (A+/T–) E4 patients showed a stable MMSE (0.10 ± 0.35) respect to clinical progression showed by (A+/T+) E3 (–3.74 ± 0.91; $p = 0.006$), (A+/T+) E4 (–5.06 ± 1.48; $p = 0.001$) but not (A+/T–) E3 patients (–1.67 ± 0.61; $p = 0.400$). At 24 months we found also a statistically significant difference for clinical progression between (A+/T–) E3 and (A+/T+) E4 ($p = 0.044$) (Fig. 2B).

DISCUSSION

The adoption of the NIA-AA consensus guidelines associated with the *APOE* genotype allowed us to reveal surprising results on the role of neuroinflam-

Uncorrected Author Proof

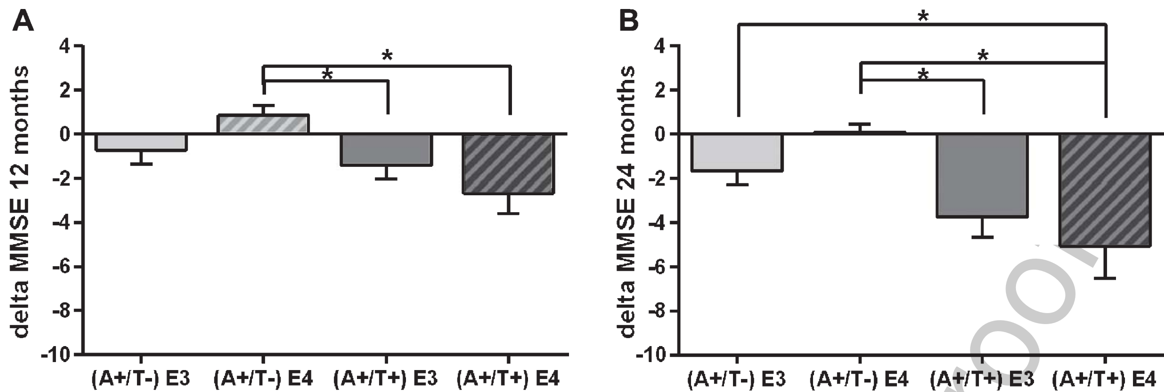

Fig. 2. Clinical progression evaluated as delta MMSE score at 12 and 24 months with respect to baseline. A) At 12 months, A+/T– patients showed substantial clinical stability. In particular, a significant difference was found between (A+/T–) E4 patients and both A+/T+ patients whatever the *APOE* genotype. B) At 24 months, (A+/T–) E4 patients still showed clinical stability as opposed to A+/T+ patients. Interestingly, (A+/T–) E3 patients showed a slight clinical worsening, with a significant difference respect to (A+/T+) E4 patients. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

mation in AD. Our study showed that CSF cytokines' levels in A+/T+ patients are similar to that of controls, regardless the *APOE* genotype. Moreover, we found that a specific pattern of AD related pathology, the (A+/T–) in *APOE4* carriers was associated with significantly increased levels of CSF IL-4, IL-6, IL-8, and G-CSF. Cognitive decline progression of this subgroup of patients, measured over a period of 24 months, appeared significantly more preserved than that observed in the other groups. Our data lead us to suggest a relationship between *APOE4* status and Aβ pathology in the absence of tau-related neurodegeneration possibly linked to a subset of cytokines exerting a protective action on the progression of cognitive symptoms.

ApoEs are lipoprotein produced and released by astrocytes, mainly involved in lipid transport to neurons and useful to support neuronal metabolism, synaptic plasticity, and neuronal repair in cases of injuries. In humans, *APOE4* is the major risk factor for developing AD [26] and in healthy individuals is associated with a reduced Aβ clearance and to a potential development of pathological changes responsible for cognitive decline [27]. Experimental AD settings have shown that ApoE4 increases Aβ synthesis, reduces its clearance, and increase Aβ dependent apoptosis of neurons [28, 29]. Such conditions inevitably lead to increase the Aβ burden and favor the hampering of cortical neurotransmission. However, our data suggest that *APOE4* genotype, associated with an isolated Aβ pathology, favors the synthesis and release of cytokines from astrocytes and microglial cells that could sustain the physiological mechanisms of synaptic transmission, thus preserving from cognitive decline. Indeed, cytokines could have beneficial effects reducing Aβ burden and potentiating synaptic transmission. IL-6 is a pleiotropic cytokine able to influence synaptic functions through IL6R located on neurons [30] of glutamatergic [31], catecholaminergic and cholinergic transmission [32]. Through its interaction with excitatory pathways, IL-6 can participate to the clearance of Aβ peptides [31] reducing its presence at synapses. IL-4 is a cytokine with anti-inflammatory activity, influencing astrocytes in the synthesis of neurotrophic growth factors [33]. Moreover, in experimental settings, IL-4 has been shown to promote microglial clearance of Aβ oligomers [34]. IL-8 is a chemokine that protects neurons by both paracrine or autocrine loop [35]. In cases of Aβ pathology, IL-8 is able to inhibit Aβ-induced apoptosis and promotes synthesis and release of brain-derived neurotrophic factor protecting neuronal survival [36]. G-CSF is a growth factor involved in stimulation and maturation of blood cells. Besides that, G-CSF plays a key role in neurogenesis and differentiation during brain development and a direct influence on synaptic plasticity [37]. G-CSF can also influence neuronal activity through its receptors expressed in hippocampus and frontal cortices [38].

In this view, it is likely to suppose that ApoE4 and Aβ can interact positively in the synthesis of neuroprotective cytokines. This interaction is specific for *APOE4*, since *APOE3* patients with isolated Aβ pathology did not show the same increase of CSF cytokines. This is likely because *APOE3* is gener-

Uncorrected Author Proof

ally associated per se with higher neuronal resilience and protection from the risk to develop AD pathology [39]. Interestingly, in A+/T+ patients we did not observe any change in cytokines' level, neither pro-inflammatory nor anti-inflammatory, regardless the *APOE* genotype. Thus, in case of association between Aβ and tau pathology the possible protective effect of both *APOE4* and *APOE3* [38, 40] is hampered. In particular, our data showed a more rapid cognitive decline in A+/T+ respect to A+/T– patients. We hypothesize that in presence of persistent noxious stressors (Aβ and tau protein) the increased synthesis of ApoE by neurons may induce a neuron-specific proteolytic pathway responsible for the production of ApoE fragments with neurotoxic effects, such as mitochondrial energy impairment, increase of tau phosphorylation and cytoskeletal disruption [29]. Moreover, among A+/T+ patients, *APOE4* carriers did not exhibit higher levels of CSF cytokines, but rather a marked cognitive decline, even worse than that shown by *APOE3* patients. Indeed, it is likely that apolipoprotein E3, with a specific binding site for tau, can be protective against the excess of tau phosphorylation, which is deleterious for neuronal survival [11, 41]. Conversely, apolipoprotein E4 does not have such binding site, and therefore patients are more exposed to neurodegeneration and cognitive decline [40].

In conclusion, even if previous evidence suggests a detrimental role of neuroinflammation in AD [42], our findings indicate that the specific condition of isolated amyloidosis (A+/T–) with *APOE4* status is associated in the CNS to an increase level of cytokines able to support the physiological mechanisms of neurotransmission and to reduce the Aβ deposition [30–38], which in our patients is expressed by a significant cognitive preservation over a period of 24 months. However, in A+/T+ condition the upregulation of cytokines and chemokines is hampered regardless the *APOE* genotype, probably because in an advanced stage of neurodegeneration, neuroinflammation is no longer able to support synaptic functioning. In agreement with our results, Taipa and colleagues recently reported a significant correlation between elevated levels of proinflammatory cytokines in the CSF of patients with AD and the cognitive status, suggesting that a stronger inflammatory response leads to a better clinical progression [21]. These findings at a first glance may seem to be in conflict with previous literature, reporting a pathological chronic activation of the innate immune system, with altered production of cytokines [43, 44] associated with the neurodegenerative processes of dementias. Intriguingly, these conflicting findings, as well our results, do nothing but reinforce the concept of neuroinflammation as a dynamic process that can act differently as a protective or harmful mechanism depending on the stage of disease and the genetic substrate (e.g., *APOE*).

Our study has some limitation, first of all the small sample size. Larger samples of patients and controls are needed to detect other significant difference in cytokines levels. Nevertheless, our study has the merit to measure cytokine's contents directly in the CSF of patients which have a robust diagnosis of AD pathology, supported by CSF biomarkers (Aβ, tau, p-tau), and long clinical follow-up periods.

In summary, although several studies suggest the modulation of pro-inflammatory cytokines production as a therapeutic target in AD [45, 46], the present work suggests that caution must be taken on modulate neuroinflammatory signaling to ensure that protective pathways are not compromised. Future studies are needed to disentangle the intricate role of neuroinflammation in AD to provide valuable cues for the development of more selective therapeutic strategies.

ACKNOWLEDGMENTS

This work was not supported by any grant.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/19-1250r2).

REFERENCES

[1] Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* **8**, 595-608.

[2] Skaper SD, Facci L, Zusso M, Giusti P (2018) An inflammation-centric view of neurological disease: Beyond the neuron. *Front Cell Neurosci* **12**, 72.

[3] Mosher KI, Wyss-Coray T (2014) Microglial dysfunction in brain aging and Alzheimer's disease. *Biochem Pharmacol* **88**, 594-604.

[4] Zheng C, Zhou XW, Wang JZ (2016) The dual roles of cytokines in Alzheimer's disease: Update on interleukins, TNF-α, TGF-β and IFN-γ. *Transl Neurodegener* **5**, 7.

[5] Udeochu JC, Shea JM, Villeda SA (2016) Microglia communication: Parallels between aging and Alzheimer's disease. *Clin Exp Neuroimmunol* **7**, 114-125.

[6] Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P (2009) Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *J Neuroimmunol* **210**, 3-12.

[7] Alasmari F, Alshammari MA, Alasmari AF, Alanazi WA, Alhazzani K (2018) Neuroinflammatory cytokines induce amyloid beta neurotoxicity through modulating amyloid

Uncorrected Author Proof

precursor protein levels/metabolism. *Biomed Res Int* **2018**, 3087475.

[8] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297**, 353-356.

[9] Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R (2011) Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener* **6**, 39.

[10] Iqbal K, Liu F, Gong CX (2016) Tau and neurodegenerative disease: The story so far. *Nat Rev Neurol* **12**, 15-27.

[11] Fá M, Puzzo D, Piacentini R, Staniszewski A, Zhang H, Baltrons MA, Li Puma DD, Chatterjee I, Li J, Saeed F, Berman HL, Ripoli C, Gulisano W, Gonzalez J, Tian H, Costa JA, Lopez P, Davidowitz E, Yu WH, Haroutunian V, Brown LM, Palmeri A, Sigurdsson EM, Duff KE, Teich AF, Honig LS, Sierks M, Moe JG, D'Adamio L, Grassi C, Kanaan NM, Fraser PE, Arancio O (2016) Extracellular tau oligomers produce an immediate impairment of LTP and memory. *Sci Rep* **6**, 19393.

[12] Van der Vlies AE, Verwey NA, Bouwman FH, Blankenstein MA, Klein M, Scheltens P, van der Flier WM (2009) CSF biomarkers in relationship to cognitive profiles in Alzheimer disease. *Neurology* **72**, 1056-1061.

[13] Koch G, Belli L, Giudice TL, Di Lorenzo F, Sancesario GM, Sorge R, Bernardini S, Martorana A (2013) Frailty among Alzheimer's disease patients. *CNS Neurol Disord Drug Targets* **12**, 507-511.

[14] Koch G, Di Lorenzo F, Del Olmo MF, Bonní S, Ponzo V, Caltagirone C, Bozzali M, Martorana A (2016) Reversal of LTP-like cortical plasticity in Alzheimer's disease patients with tau-related faster clinical progression. *J Alzheimers Dis* **50**, 605-616.

[15] Koch G, Esposito Z, Kusayanagi H, Monteleone F, Codecá C, Di Lorenzo F, Caltagirone C, Bernardi G, Martorana A (2011) CSF tau levels influence cortical plasticity in Alzheimer's disease patients. *J Alzheimers Dis* **26**, 181-186.

[16] Koch G, Di Lorenzo F, Loizzo S, Motta C, Travaglione S, Baiula M, Rimondini R, Ponzo V, Bonnì S, Toniolo S, Sallustio F, Bozzali M, Caltagirone C, Campana G, Martorana A (2017) CSF tau is associated with impaired cortical plasticity, cognitive decline and astrocyte survival only in APOE4-positive Alzheimer's disease. *Sci Rep* **7**, 13728.

[17] Shi Y, Yamada K, Liddelow SA, Smith ST, Zhao L, Luo W, Tsai RM, Spina S, Grinberg LT, Rojas JC, Gallardo G, Wang K, Roh J, Robinson G, Finn MB, Jiang H, Sullivan PM, Baufeld C, Wood MW, Sutphen C, McCue L, Xiong C, Del-Aguila JL, Morris JC, Cruchaga C; Alzheimer's Disease Neuroimaging Initiative, Fagan AM, Miller BL, Boxer AL, Seeley WW, Butovsky O, Barres BA, Paul SM, Holtzman DM (2017) ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* **549**, 523-527.

[18] Famenini S, Rigali EA, Olivera-Perez HM, Dang J, Chang MT, Halder R, Rao RV, Pellegrini M, Porter V, Bredesen D, Fiala M (2017) Increased intermediate M1-M2 macrophage polarization and improved cognition in mild cognitive impairment patients on ω-3 supplementation. *FASEB J* **31**,148–160.

[19] Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, Beckers L, O'Loughlin E, Xu Y, Fanek Z, Greco DJ, Smith ST, Tweet G, Humulock Z, Zrzavy T, Conde-Sanroman P, Gacias M, Weng Z, Chen H, Tjon E, Mazaheri F, Hartmann K, Madi A, Ulrich JD, Glatzel M, Worthmann A, Heeren J, Budnik B, Lemere C, Ikezu T, Heppner FL, Litvak V, Holtzman DM, Lassmann H, Weiner HL, Ochando J, Haass C, Butovsky O (2017) The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* **47**, 566-581.

[20] Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* **14**, 535-562.

[21] Taipa R, das Neves SP, Sousa AL, Fernandes J, Pinto C, Correia AP, Santos E, Pinto PS, Carneiro P, Costa P, Santos D, Alonso I, Palha J, Marques F, Cavaco S, Sousa N (2019) Proinflammatory and anti-inflammatory cytokines in the CSF of patients with Alzheimer's disease and their correlation with cognitive decline. *Neurobiol Aging* **76**, 125-132.

[22] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[23] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.

[24] Di Lorenzo F, Motta C, Bonnì S, Mercuri NB, Caltagirone C, Martorana A, Koch G (2019) LTP-like cortical plasticity is associated with verbal memory impairment in Alzheimer's disease patients. *Brain Stimul* **12**, 148-151.

[25] Martorana A, Esposito Z, Di Lorenzo F, Giacobbe V, Sancesario GM, Bucchi G, Bonnì S, Bernardini S, Sorge R, Sancesario G, Bernardi G, Caltagirone C, Koch G (2012) CSF levels of Aβ1-42 relationship with cholinergic cortical activity in Alzheimer's disease patients. *J Neural Transm* **119**, 771-778.

[26] Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. *Nat Rev Neurol* **9**, 106-118.

[27] Bunce D, Fratiglioni L, Small BJ, Winblad B, Bäckman L (2004) APOE and cognitive decline in preclinical Alzheimer disease and non-demented aging. *Neurology* **63**, 816-821.

[28] Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, Sartorius LJ, Mackey B, Olney J, McKeel D, Wozniak D, Paul SM (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* **97**, 2892-2897.

[29] Mahley RW, Weisgraber KH, Huang Y (2006) Apolipoprotein E4: A causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A* **103**, 5644-5651.

[30] Gruol DL (2015) IL-6 regulation of synaptic function in the CNS. *Neuropharmacology* **96**, 42-54.

Uncorrected Author Proof

[31] Hettinger JC, Lee H, Bu G, Holtzman DM, Cirrito JR (2018) AMPA-ergic regulation of amyloid-β levels in an Alzheimer's disease mouse model. *Mol Neurodegener* **13**, 22.

[32] Zalcman S, Green-Johnson JM, Murray L (1994) Cytokine-specific central monoamine alterations induced by interleukin-1, -2 and -6. *Brain Res* **643**, 40-49.

[33] Gadani SP, Cronk JC, Norris GT, Kipnis J (2012) IL-4 in the brain: A cytokine to remember. *J Immunol* **189**, 4213-4219.

[34] Shimizu E, Kawahara K, Kajizono M, Sawada M, Nakayama H (2008) IL-4-induced selective clearance of oligomeric beta-amyloid peptide (1-42) by rat primary type 2 microglia. *J Immunol* **181**, 6503-6513.

[35] Franciosi S, Ryu JK, Choi HB, Radov L, Kim SU, McLarnon JG (2006) Broad-spectrum effects of 4-aminopyridine to modulate amyloid beta1-42-induced cell signaling and functional responses in human microglia. *J Neurosci* **26**, 11652-11664.

[36] Ashutosh, Kou W, Cotter R, Borgmann K, Wu L, Persidsky R, Sakhuja N, Ghorpade A (2011) CXCL8 protects human neurons from amyloid-β-induced neurotoxicity: Relevance to Alzheimer's disease. *Biochem Biophys Res Commun* **412**, 565-571.

[37] Sopova K, Gatsiou K, Stellos K, Laske C (2014) Dysregulation of neurotrophic and haematopoietic growth factors in Alzheimer's disease: From pathophysiology to novel treatment strategies. *Curr Alzheimer Res* **11**, 27-39.

[38] Marsland AL, Gianaros PJ, Kuan DC, Sheu LK, Krajina K, Manuck SB (2015) Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain Behav Immun* **48**, 195-204 (2015).

[39] Aboud O, Mrak RE, Boop F, Griffin ST (2012) Apolipoprotein epsilon 3 alleles are associated with indicators of neuronal resilience. *BMC Med* **10**, 35.

[40] Lee YM, Ha JK, Park JM, Lee BD, Moon E, Chung YI, Kim JH, Kim HJ, Mun CW, Kim TH, Kim YH (2016) Impact of Apolipoprotein E4 polymorphism on the gray matter volume and the white matter integrity in subjective memory impairment without white matter hyperintensities: Voxel-based morphometry and tract-based spatial statistics study under 3-Tesla MRI. *J Neuroimaging* **26**, 144-149.

[41] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 1977-1981.

[42] Heneka MT, Kummer MP, Latz E (2014) Innate immune activation in neurodegenerative disease. *Nat Rev Immunol.* **14**, 463-477.

[43] Sochocka M, Diniz BS, Leszek J (2017) Inflammatory response in the CNS: Friend or foe? *Mol Neurobiol* **54**, 8071-8089.

[44] Blennow K, Hampel H, Weiner M, Zetterberg H (2010) Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol.* **6**, 131-144.

[45] Parachikova A, Vasilevko V, Cribbs DH, LaFerla FM, Green KN (2010) Reductions in amyloid-beta-derived neuroinflammation, with minocycline, restore cognition but do not significantly affect tau hyperphosphorylation. *J Alzheimers Dis* **21**, 527-542.

[46] Shi JQ, Shen W, Chen J, Wang BR, Zhong LL, Zhu YW, Zhu HQ, Zhang QQ, Zhang YD, Xu J (2011) Anti-TNF-α reduces amyloid plaques and tau phosphorylation and induces CD11c-positive dendritic-like cell in the APP/PS1 transgenic mouse brains. *Brain Res* **1368**, 239-247.

Uncorrected Author Proof