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Short Communication

Interleukin-10 Production in Response to Amyloid-β Differs between Slow and Fast Decliners in Patients with Alzheimer's Disease

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Abstract. We investigated IL-10 and IL-6 production in amyloid-β (Aβ) stimulated peripheral blood mononuclear cells (PBMCs) in twenty Alzheimer's disease (AD) patients with slow progression, eleven with fast progression, and twenty age-matched controls. Promoter polymorphisms in IL-10 (position −592, −819, −1082), IL-6 (−174), transforming growth factor-β1 (TGF-β1) (−10, −25), interferon-γ (IFN-γ) (−874), and tumor necrosis factor-α (TNF-α) (−308) genes were analyzed. IL-10 production after Aβ stimulation was high in PBMCs from slow decliners and almost completely abrogated in fast decliners. Association between AA IFN-γ low-producing genotype and fast progression was demonstrated. Investigations in a larger sample will clarify these findings.

Keywords: Alzheimer's disease, disease progression, IFN-γ, interleukin-6, interleukin-10, peripheral blood mononuclear cells, single nucleotide polymorphisms

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INTRODUCTION

Alzheimer's disease (AD) is a multifactorial disease and there is increasing evidence that the immune system plays a critical role in the clinical symptoms [1–4]. Interleukin-10 (IL-10) is well characterized as an anti-inflammatory cytokine, with potent suppressive effects in preventing autoimmune disease [5]. The main function of IL-10 seems to be to limit and terminate the inflammatory signal in cells such as monocytes and macrophages [6, 7]. Studies have shown that IL-10 is associated with cancer, AD, systemic sclerosis, type 2 diabetes, ischemic stroke, atherosclerosis, cardiovascular disease, ankylosing spondylitis, asthma, rheumatoid arthritis, and prostate hyperplasia [8–16]. Although considered to be immunosuppressive in function, the use of IL-10 as a potential therapeutic has given mixed results, suggesting a more complex role in immune regulation. The effect of IL-10 on B cells may be stimulatory rather than suppressive [17, 18].

To date, −1082AA low IL-10 producer genotype was associated with higher risk of developing AD, whereas −1082GG high IL-10 producer genotype frequency is higher in centenarians [8, 19–23]. A study following subjects with mild cognitive impairment (MCI) has shown that MCI converting to AD showed a higher frequency of allele A and −1082AA IL-10 genotype compared with stable MCI or those who convert to vascular dementia suggesting a role of −1082AA polymorphism in converting from MCI to AD [23]. *Ex vivo* studies confirmed this link between AD and decreased IL-10 production [20, 21].

The objective of our study was to further investigate the role of IL-10 in AD, in combination with other cytokine gene polymorphisms (IL-6, IL-10, TNF-α, TGF-β1, and IFN-γ).

MATERIALS AND METHODS

A total of 51 individuals were enrolled in the study: 20 healthy elderly (controls) and 31 AD patients (Table 1). Subjects diagnosed with AD fulfilled the criteria of dementia and AD defined by NINCDS-ADRDA [24]. All individuals were Caucasians living in Milan or Paris and belonged to larger populations of outpatients.

The criteria for the diagnosis of normal cognition were: 1) no active neurological or psychiatric disorder; 2) no ongoing medical problems or related treatments interfering with cognitive function; 3) a normal neurological exam; 4) no psychoactive medications; and 5) the ability to live and function independently in the

Table 1
Demographic and clinical characteristics of Alzheimer's patients and age-matched controls

		AD patients			
	Controls ($n = 20$)	AD slow ($n = 20$)	AD fast ($n = 11$)	Total ($n = 31$)	p value
Demographic data					
Age, median (range)	78.2 (63.7–96.2)	80.5 (71.2–88.0)	78.8 (74.5–86.5)	79.5 (71.2–88.0)	ns
Ethnicity, *n* (%)					
African	0 (0%)	0 (0%)	1 (9%)	1 (3%)	ns
Caucasian	20 (100%)	20 (100%)	10 (91%)	30 (97%)	
Alzheimer's disease data					
Age of AD onset, median (range)	NA	77.0 (66.4–86.3)	77.4 (70.6–83.6)	77.0 (66.4–86.3)	ns
Duration of the disease before recruitment (y), median (range)	NA	2.8 (1.4–9.4)	2.9 (1.4–6.4)	2.9 (1.4–9.4)	ns
MMSE score**, median (range)	30 (26–30)	21 (8–25)	14 (5–21)	17 (5–25)	a: 4.66E–7 b: 2.81E–6 c: 4.33E–5 d: 0.001
Calculated ΔMMSE over 2 y, median (range)	0.0 (–0.9 – 0.9)	–0.5 (–3.5 – 1.2)	–7.2 (–16.1 – –3.8)	–2 (–16.1 – 1.2)	d: 5,6E–6
Unknown	18/20	0	0	0	

AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; NA, not applicable; ns, not significant; y, years. AD slow and AD fast stand for AD patients displaying slow and fast disease progression, respectively. Regarding statistical analysis, demographic and clinical data were analyzed using Kruskall-Wallis test for multiple comparisons or Mann–Whitney rank sum test for comparing two groups. Comparison of AD treatment was done with χ^2 test, Fisher's exact test, or the Freeman-Halton extension of the Fisher exact probability test. a, total age-matched controls to total AD patients comparison, b, total age-matched controls to AD slow comparison, c, total age-matched controls to AD fast patients comparison, d, AD slow to AD fast patients comparison. MMSE score at the end of the follow up period.

community. Individuals affected by cancer or cardiovascular diseases were excluded. At recruitment, none of the subjects showed clinical signs of inflammation. All participants and their relatives gave informed consent and the respective ethics committees approved the study protocol.

At baseline, physical, neurological, and neuropsychological examinations were performed for all patients together with clinical history, computed tomography, or magnetic resonance imaging scan, and cognitive testing using the Mini-Mental State Examination (MMSE). Laboratory analyses included routinely biochemical tests. After a two-year period follow-up, AD patients were retrospectively evaluated and AD progression rate was calculated by using deltaMMSE score (MMSE at recruitment - MMSE after the two-year follow-up period). Patients were categorized as slow-progressing AD if deltaMMSE ≤ 3 points and no appearance of new cognitive disorders (AD slow) or fast-progressing AD if deltaMMSE ≥ 5 points and appearance of other AD related disorders (AD fast). Blood from all individuals was collected at the same time in the morning and employed for biochemical tests and PBMCs isolation. PBMCs were cultured in medium, medium+LPS (1 ng/ml), or medium+pool of three Aβ peptides: 1 μg/ml of fragment (1–16), 10 μg/ml of fragment (25–35), and 25 μg/ml of fragment (1–40) (Innovagen, Lund, Sweden). IL-10 production was measured with ELISA (Ready-SET-Go! test; eBioscience, Paris, France).

Statistical analyses were conducted using softwares R and SPSS. As samples were independent and not normally distributed, Kruskall-Wallis test for multiple comparisons, Mann–Whitney rank sum test for comparing two groups and Wilcoxon sign test for intergroup comparison (of two medians observed in paired series) were used. For proportion comparison, χ^2 test (if $N > 5$ or at least 80% of the cells have an expected frequency of 5 or greater, and that no cell has an expected frequency smaller than 1.0) and Fisher's exact test (if $N < 5$ in the case of a 2×2 contingency table) or the Freeman-Halton extension of the Fisher exact probability test (if $N < 5$ in the n × m contingency table with n and $m > 2$) were used.

RESULTS

The median (Table 1) and mean MMSE at the blood sample collection was higher among AD slow than AD fast (mean values: 19.6 ± 4.6, and 12.3 ± 5.4, $p < 0.05$). Frequencies of patients with or without AD targeted therapies were statistically similar between the two groups (data not shown).

The analysis did not show any statistically significant changes in biochemical analysis in relation to AD and/or progression. Co-morbid conditions were not different between controls and AD patients, and between controls and AD slow or AD fast (data not shown). Significant increase of IL-10 production in PBMCs after LPS-stimulation was showed in AD patients and controls with no significant differences between the groups (controls: 20.9 ± 6.0 versus 484.6 ± 248.9; $p = 7.6\ 10^6$, total AD patients: 19.4 ± 6.8 versus 704.4 ± 238.0; $p = 1.8\ 10^6$, AD slow: 23.2 ± 5.7 versus 671.0 ± 285.5; $p = 9.6\ 10^5$, AD fast: 16.2 ± 4.9 versus 797.3 ± 223.4; $p = 0.002$, Fig. 1A). Aβ-stimulated IL-10 production analysis showed a significant increase of antigen-specific IL-10 production compared with resting production in AD with slow progression (40.7 ± 13.7 versus 59.0 ± 27.0; $p = 0.004$, Fig. 1B). Interestingly, AD fast did not show any difference, suggesting a lack of response to Aβ stimulus (39.7 ± 14.4 versus 42.2 ± 22.4; $p = 0.94$).

No significant differences were shown in IL-10 and IL-6 plasma concentrations between AD and controls, probably due to the small sample size (data not shown).

Despite there being no significant differences in genotype and allele frequencies of –1082 G/A ($p = 0.61$ and $p = 0.33$, respectively), –819 C/T and –592 C/A SNPs ($p = 0.47$ and $p = 0.24$, respectively), there is a trend toward AD patients having a higher frequency of the low/intermediate IL-10-producing genotypes (−1082 AA/GA: 77%) and lower frequency of −1082GG genotype (23%) compared to controls (65% and 35%, respectively) [19, 20, 23, 25, 26]. AD fast showed a lower frequency of high IL-10 producing genotype compared to AD slow (25.0% versus 18.2%). In addition, GG genotype could confer a slower progression rate of the disease.

Analysis of the IFN-γ genotype distribution between controls and AD showed the highest frequency of AA genotype (81.8%), associated with decreased IFN-γ levels [27] in AD fast ($p = 0.003$) compared to the other groups (25% in AD slow and 25% in controls). Similarly, the A allele was significantly more represented among AD fast ($p = 0.042$) (81.1% versus 55% in AD slow and 50% in controls). Distribution of genotype and allele frequencies of IL-6 (−174), TNF-α (−308), and TGF-β1 (−10 and −25) did not differ (data not shown).

Despite the not significant result obtained for the ApoE ε4 distribution ($p = 0.08$), the groups show clear differences in the numbers of ε4 carriers (31.6%,

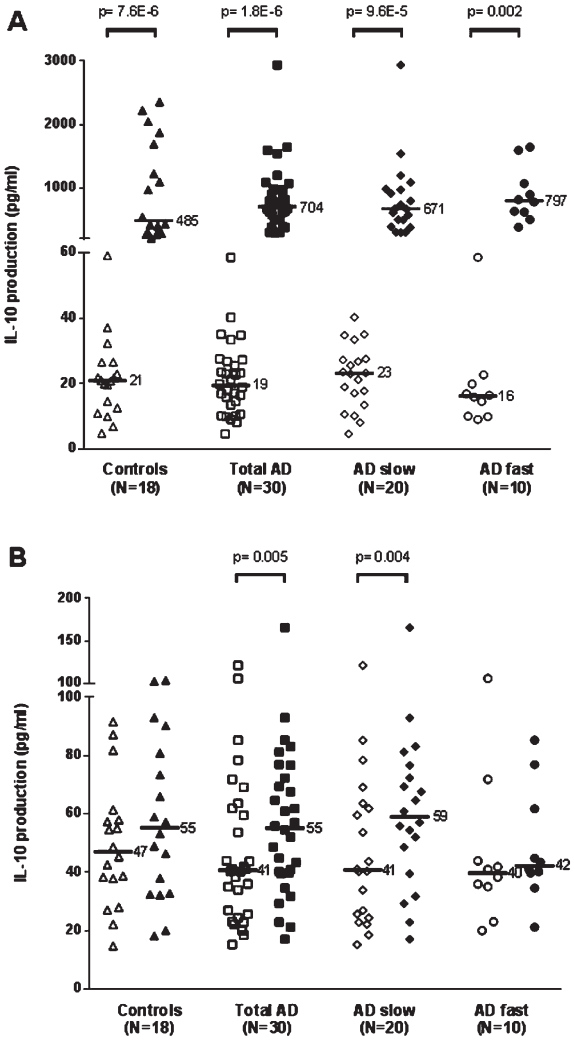

Fig. 1. *Ex vivo*, LPS- and Aβ-induced IL-10 production by PBMCs of AD patients and age-matched controls. PBMCs (3.10^6 cells / ml) from AD patients ($n = 30$) and age-matched controls ($n = 18$) were cultured either in complete medium alone (open symbol), supplemented with 1 ng/ml of LPS (full symbol, A) during two days or supplemented with a pool of three Aβ peptides: 1 μg/ml of fragment (1-16), 10 μg/ml of fragment (25–35), and 25 μg/ml of fragment (1–40) (full symbol, B) during five days. Results have been showed as scatter plot and the median value is indicated by a black plain line. Kruskall-Wallis and Mann-Whitney U-test were applied for intergroup comparison. For intragroup comparison of IL-10 production, Wilcoxon sign test was used as it was comparison of two medians observed in paired series.

55.6%, and 60% of ε4 carriers, respectively in controls, AD slow, and AD fast).

We report a significantly increased Aβ specific PBMCs IL-10 and IL-6 productions (Fig. 1 and Supplementary Material) in AD slow ($p = 0.004$ and $p = 0.01$ for IL-10 and IL-6). Abrogation of IL-10 specific production has been demonstrated in AD fast suggesting impaired response to Aβ stimulus in these patients.

DISCUSSION

In accordance with our findings, some authors have reported an increase of IL-10 and IL-1ra, which should balance the higher *in vitro* production of pro-inflammatory cytokines [28, 29]. However, some studies demonstrated a reduction of both pro- and anti-inflammatory cytokines [30, 31], suggesting a general impairment of immune functions in AD. Other studies demonstrated a decrease of IL-10 and an increase of pro-inflammatory cytokines IL-1β, IL-6, and IFN-γ [21, 32–34]. This variability could be explained by methodological differences among studies, including inclusion criteria of both AD patients and healthy controls and methods of mitogen- or Aβ-stimulation. Ours is the first study in which the rate of AD disease progression has been taken into account.

Data on IL-10 −1082 polymorphism suggested an association with AD risk indicating protective effect of −1082GG genotype [19, 26]. Our data suggest that GG genotype (associated with highest IL-10 production) could confer additional protection from a fast progression rate of AD.

Studies have shown that IFN-γ −874 TA polymorphism does not represent a risk factor for AD [35–39]. However, our data suggests that this polymorphism could be involved in disease progression, with high IL-10 production and high IFN-γ genotype [27] conferring a slower AD progression. The presence of high producer T allele is associated with highest IFN-γ mRNA expression [40] and blood levels both at baseline and after PBMCs stimulation [41]. Our results show that the frequency of the low producer A allele of IFN-γ is increased in patients with fast progression.

Our data suggest that fast AD progressing lack ability to mount effective anti-inflammatory response to Aβ contributing to the de-regulation of their immune system. Elucidation of these mechanisms using larger study group may shed new light on the potential avenues to manage the disease progression.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/14-2832r1).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-142832.

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