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ORIGINAL ARTICLE

Cytokine polymorphisms and Alzheimer disease: possible associations

G. Ribizzi · S. Fiordoro · S. Barocci · E. Ferrari · M. Megna

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Abstract Alzheimer's disease (AD) is a degenerative dementia characterized by typical, destructive alterations of neurons (neurofibrillary tangles and amyloid plaques), and glial proliferation. Cytokine-driven inflammatory environment can contribute to the pathogenesis and/or progression of the disease. The aim of the study was to evaluate and compare genotypic and allelic polymorphisms of 13 cytokine genes in 19 Caucasoid AD patients with medium–high level of dementia (assessed by an MMSE < 24) and 20 normal controls affected by non inflammatory neuropsychiatric disease. Polymorphisms in the genes of IL-lA, IL-lB, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-G, TGF-β, TNF-α, and of the cytokine receptors IL-lR, IL-IRA, IL-4RA were investigated. APO-E and ACE gene polymorphisms were carried out in the patient's group only to evaluate a possible association with known genetic risk factors for AD. A highly significant presence of some alleles belonging to anti-inflammatory cytokine genes was found; particularly the C allele for the -590 promoter and T allele for the -1098 promoter of IL-4 appeared in a significantly higher percentage as compared with controls ($P < 0.0006$ and $P < 0.0005$, respectively), while a lesser significance was observed for the allele C of the -819 promoter of IL-10 ($P < 0.03$). Finally, in the group of demented patients for the APO-E gene we found a statistically significant presence of the E4 allele, whereas no difference was found for the polymorphisms of the ACE gene. Our observations corroborate the possible presence of a pro-inflammatory environment in AD patients, partly sustained by the low expression of anti-inflammatory cytokine genes when defined alleles are present. Large cohort studies are necessary in order to assess the real association of some cytokine alleles or haplotypes with AD.

Keywords Alzheimer · Cytokine · Genetic polymorphisms

G. Ribizzi (✉)
Department of Neurology, Ospedale S. Martino, L.go R. Benzi, 10, 16100 Genoa, Italy
e-mail: gribizz1@tin.it

S. Fiordoro · S. Barocci · E. Ferrari
Immunology Service, Ospedale S. Martino, L.go R. Benzi, 10, 16100 Genoa, Italy

M. Megna
Department of Neurology, University of Bari, P.zza G. Cesare, 11, 70124 Bari, Italy

Introduction

Alzheimer's disease (AD) is a dementia characterized by neuronal loss, atrophy, gliosis, and clinically by progressive cognitive impairment. The neuropathological hallmarkers are β-amiloid plaques (BAP) and neurofibrillary tangles (NFT). In the past years several experimental evidences, both in vitro and in vivo suggested a possible involvement of the immune system in the pathogenesis and/or in the progression of the disease. In this context, a particular role seems to be played by inflammatory cytokines such as IL-1, IL-6, and TNF-α. Cytokines seem critical in the pathobiology of AD as their production can be amplified in an inflammatory atmosphere, following primed glial activation by a yet unknown trigger [1]. This cytokine inflammatory environment is also supported by experimental model demonstrating that cytokine modulating therapies can improve AD pathology [1].

Particularly, previous studies on AD showed an activation of the immune system as demonstrated by the

following: (1) An increased secretion of IL-1B, IL-6 and TNF-A and IL-10 by in vitro LPS polysaccharide AD lymphocytes as compared with controls [2]; (2) serum and antineuronal autoantibodies [3]; (3) presence in the amyloid plaques of cytokines, complement fragments, proteases, and acute phase proteins. Moreover, AD studies of genetic association, in addition to well-known $\varepsilon 4$ allele of APO E gene, showed significant associations of the disease with some cytokine genotypes such as TT for IL-1A, IL-1B [4, 5] and chymotrypsin [6], or AG 99 for TNF-A.

Moreover, experimental studies on microglial cells stimulated with 1–42 peptide of β-amyloid (Aβ), showed, using microarray technique, a high transcription level of many pro-inflammatory cytokine genes (TNF-A, MCP-1, IL-8, TGF-B) [7, 8] and, on the contrary, pro-inflammatory cytokines and growth factors (CSF) induced the transcription of the promoter of amyloid precursor protein (APP) [9]. Considering the relative low quantity of studies about multiple cytokine gene polymorphisms in AD, we were prompted to study the polymorphisms of several cytokine genes in an AD group of patients and in a control healthy group. Finally, we explored the presence of AD well-known genetic risk factor, like the APO-E4 allele [10], and, more recently, the I allele of the ACE gene [11], in order to confirm the good quality of selection of our patients and evaluate the possible association of these alleles with the studied cytokine alleles or genotypes.

Methods

The following groups were analyzed:

1. 19 Caucasoid patients with NINCDS-ADRA [12] diagnosis of AD, whose characteristics are summarized in Table 1. No patients were treated with non steroidal antiinflammatory drugs (NSAID), salicylate or Ach-ase inhibitors.
2. 20 Caucasoid controls, matched by age and gender, with a normal Mini Mental State Examination (MMSE), affected by non inflammatory neuropsychiatric disease (headache, depression). The patients and the control group did not show either symptoms or signs of inflammatory process.

In the AD group we performed blood examinations to exclude internal or inflammatory diseases causing a dementia (B12, folic acid, thyroid hormones, PCR, C3, C4 and ANA): all tests were within normal values (data not shown).

CT scan was performed in the AD group to exclude any other neurological causes of dementia.

All patients and controls gave informed consent.

Table 1 Study group characteristics

	AD	Controls
N	19	20
Age (media)	62–84 (76.27)	60–82 (73.1)
Sex F/M (%)	16/6 (73.3/22.7)	16/4 (80/20)
MMSE media	20.26	28.6
Years of disease (±SD)	4.5 (3–8)	
Associated disease (*N*)	Hypertension (6), Renal failure (1), Cardiac arrythmia (1), Parkinson (1)	

Genetic analysis

Genomic DNA was extracted according the methods of Barocci et al. [15]. Twenty-two genomic polymorphisms of the following cytokines IL1-A, IL1-B, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-G, TGF-B, TNF-A and of the cytokine receptors IL-1R, IL-1 RA, IL-4 RA, were analyzed in both groups by PCR-SSP methodology according Uboldi De Capei [13]; the specificity of these polymorphisms is reported out in the results.

APO-E and ACE polymorphisms were carried out to evaluate a possible association with known genetic risk factors for AD according to previously described methods [14, 15].

Statistical analysis

Allelic frequencies were evaluated by direct gene count. Statistically significant differences between the allele frequencies of every polymorphism in the two groups were evaluated by a chi-square test of the PSSP package (Statistical Product and Service Solution Package; Chicago, IL) with a significant set value at $P < 0.05$. Hardy–Weinberg equilibrium was verified by using Pearson's test. Differences in genotype frequencies in both groups were calculated using the Monte Carlo algorithm in the PSSP software.

Results

The statistical analysis of genotypic distribution did not show any significant difference between the two groups relating to the following cytokine polymorphisms: IL-1R (pst 1970), IL-1RA (mspa1 1100), IL4-RA (+1902), IL-12 (−1188), IFN-G (UTR 5644), TGF-B (codon 10), TGF-B (codon 25), IL-2 (−330), IL-2 (+166), IL-6 (−174), IL-6 (nt 565), TNF-A (−308), TNF-A (−238), IL-4 (−33) e IL-10 (−592).

Table 2 Genotype distributions of IL-1A, IL-1B, IL-4, and IL-10 polymorphisms in AD and controls

Cytokine	Position	Genotype *N* (%) AD (*N* = 19)	Genotype *N* (%) Controls (*N* = 20)	*P* value
IL-1A	−889	CC 12 (63)	CC 7 (35)	NS
		CT 3 (16)	CT 10 (50)	0.04
		TT 4 (21)	TT 3 (15)	NS
IL-1B	−511	CC 5 (26)	CC 3 (15)	NS
		CT 14 (74)	CT 12 (60)	NS
		TT 0 (0)	TT 5 (25)	0.04
IL-1B	+3962	CC 5 (26)	CC 3 (15)	NS
		CT 14 (74)	CT 12 (60)	NS
		TT 0 (0)	TT 5 (25)	0.04
IL-4	−590	CC 15 (79)	CC 4 (20)	0.00035
		CT 2 (10)	CT 13 (65)	0.0003
		TT 2 (11)	TT 3 (15)	NS
IL-4	−1098	GG 0 (0)	GG 9 (45)	0.001
		GT 2 (11)	GT 6 (30)	NS
		TT 17 (89)	TT 5 (25)	0.000068
IL-10	−1082	AA 6 (32)	AA 7 (35)	NS
		GA 5 (26)	GA 12 (60)	0.053
		GG 8 (42)	GG 1 (5)	0.008
IL-10	−819	CC 16 (84)	CC 10 (50)	0.04
		CT 2 (11)	CT 5 (25)	NS
		TT 1 (5)	TT 5 (25)	NS

NS not significant

Table 2 shows the statistically significant differences of the following genotypes in the two groups: CT −889 of IL-1A ($P = 0.04$); TT −511 ($P = 0.04$) and TT +3962 ($P = 0.04$) of IL-1B; CC ($P = 0.00035$) and CT −590 ($P = 0.0003$) of IL-4, GG ($P = 0.001$) and TT −1098 ($P = 0.000068$) of IL-4, GA ($P = 0.053$) and GG −1082 ($P = 0.008$) of IL-10, and CC −819 ($P = 0.04$) of IL-10.

The allelic frequencies did not show any significant difference for the following polymorphisms: IL-1A (−889), IL-1B (−511), IL-1B (−3962), IL-1R (pst 1970), IL1-RA (mspa1 1100), IL4-RA (+1902), IL-12 (−1188), IFN-γ (UTR 5644), TGF-B (codon 10), TGF-B (codon 25), IL-2 (−330), IL-2 (+166), IL-6 (−174), IL-6 (nt 565), TNF-A (−308), TNF-A (−238), IL-4 (−33), IL-10 (−592), IL-10 (−819), IL-10 (−1082).

Table 3 lists the statistically significant differences for the allelic frequencies between the AD and the control group.

Finally, in the AD group we did not find any increased frequency of allele or genotype of the pro-inflammatory cytokine gene panel; on the contrary, the anti-inflammatory cytokine gene set in the AD population showed a raised significant presence of the following genotypes and alleles: CC −590 ($P < 0.00035$) and TT −1098 ($P < 0.000068$) of IL-4, GG-1082 ($P < 0.008$) and CC −819 ($P < 0.04$) of IL-10, C −590 ($P < 0.04$), and T-1098 ($P < 0.00043$) of IL-4.

Statistically, in the group of demented patients, no difference was evident for the I allele of the ACE gene, whilst we confirmed the prevalence of $\varepsilon 4$ allele of the APO-E gene ($P < 0.05$) (Table 4).

Concluding, the AD group confirmed a raised significant presence of the −819 C allele of the IL-10 promoter, which was typically associated with low producers of IL-10 [16,

Table 3 Allelic polymorphisms of IL-4 promoters

Cytokine	Position	Allele *N* (%) AD (*N* = 19)	Allele *N* (%) Controls (*N* = 20)	*P* value
IL-4	−590	C 16 (84)	C 10 (50)	0.04.
		T 3 (16)	T 10 (50)	0.04
IL-4	−1098	G 1 (5)	G 12 (60)	0.00043
		T 18 (95)	T 8 (40)	0.00043

Table 4 Alleles of ACE e APO-E in AD and controls

GENE	*N* = 19 (%) AD	*N* = 20 (%) Controls	*P* value
ACE	Allele I = 6 (31.6%)	Allele I = 11 (55%)	NS
	Allele D = 13 (68.4%)	Allele D = 9 (45%)	NS
APO-E	Allele 2 = 1 (5.2%)	Allele 2 = 1 (5%)	NS
	Allele 3 = 9 (47.4%)	Allele 3 = 16 (80%)	0.028
	Allele 4 = 7 (47.4%)	Allele 4 = 3 (15%)	0.048

18]. Moreover, −1098 TT and −590 CC genotypes and the corresponding T and C alleles of the IL-4 gene were more represented in the AD group; in asthma and rheumatoid arthritis these alleles were associated with low production of Il-4 and IgE [17].

Discussion

AD experimental models and neuropathological findings have previously shown the presence of inflammatory cells and products within the active neuritic plaque; the inflammatory circle is induced by the 1–42 peptide of the amyloid β protein which stimulates microglial cells to secrete proinflammatory cytokines including TNF-A, IL-6, Il-1B, MCP-1, and cytotoxic substances represented by nitric oxide. This activation produces and maintains an inflammatory environment in the neuritic plaque that can amplify the neurodysfunction/neurodegeneration previously induced in the brain parenchyma by an unknown, yet triggering cause. In the neuritic plaque is also present a secretion of the anti-inflammatory cytokines IL-4 and IL-10 which can modulate or inhibit the Aβ-induced secretion of Il-1B, Il-6 and TNF-A [19]. Moreover in AD, the in vitro stimulation of microglial cells and monocytes by Aβ or LPS resulted in the pro-inflammatory cytokine gene expression and the subsequent secretion of corresponding cytokines [20].

Serum concentration of the various cytokines can be influenced by allelic/genotypic pattern [18, 21], and in AD, to date, no cytokine allele or genotype has been shown to be clearly associated with a raised susceptibility to the disease. In addition, various studies have shown that in brain parenchyma the response to the antigen stimulation, which can be carried out by Th1 lymphocytes with secretion of IFN-G or TNF-A or by Th2 with Il-4 and IL-10 production, can also be regulated, in addition to the antigen nature, by the presence of defined alleles in the cytokine genes [22].

In relation to our study, we are aware that the above results have been obtained from a small number of patients which greatly reduces the statistical significance of the results. We have tried to overcome this limit by analyzing a high number of cytokines, both with inflammatory and anti-inflammatory properties, in order to evaluate the presence of possible differences between AD and control group. Moreover, AD patients have been selected with very stringent criteria aimed at ruling out the influence of any extra cerebral inflammatory environment or background and strengthening the power of the analysis. This latter approach has made very difficult the enrolment of a high number of patients. However, our results show a highly significant presence of some alleles belonging to the anti-inflammatory cytokine genes IL-4 and IL-10: particularly, the C allele for the −590 promoter and the T allele for the −1098 promoter of IL-4 appeared expressed in a significant higher percentage as compared with controls. These promoter regions are important to induce and begin the transcription of IL-4 gene [23].

The T allele in the −590 position of IL-4 gene has been linked to an increased transcription of IL-4 mRNA and in turn to IgE synthesis [21, 23]: as a matter of fact, this allele is present more in patients with bronchial asthma [17] and atopy [24].

On the other side, we hypothesize the C allele at −590 site in AD patients could in turn be linked to a reduced transcription of the IL-4 in the neuritic plaque with the consequent reduction of antiinflammatory Th2 effect of the IL-4 gene [21]. The lack of IL-4 assay in our patients does not permit concluding in favor of reduced secretion and/or concentration of circulating or in situ (i.e. in the plaque) IL-4 in C allele carriers, and we believe that such an assay could be very useful for supporting the hypothesis that the high frequency of C allele in AD patients could cause a down-regulation of anti-inflammatory cytokines within the neuritic plaques [19].

The 1098 G/T promoter of IL-4 gene is associated neither with IgE synthesis nor with defined disease [17]. However, the nucleotidic shift near the 1079–86 TATA box promoter region, which is known to play a key role in gene transcription [25], might be important to lower gene transcription.

Indeed, the reduced presence of the G allele in our AD patients, as already shown for juvenile idiopathic arthritis (an autoimmune Th-1 driven disease) [26], could also be linked to a reduced transcription of the IL-4 gene.

Although our genetic findings could suppose a possible susceptibility toward a pro-inflammatory environment in AD, which is sustained by a low expression of anti-inflammatory cytokine genes, it is important to underline that our results could not apply to AD patients belonging to a different ethnic groups, as the ethnic characteristics can influence the studied polymorphism. In fact, in healthy Russians, differently from other Caucasoid populations, the G allele in the G/T−1098 polymorphism of IL-4 gene is highly prevalent (92%) [17]. A typical example of different results observed in AD patients according to the different ethnic background is represented by the defined haplotype 174-IL6C/1082-IL10A which in Italians is associated with a raised production of inflammatory cytokines and higher presence of AD. On the contrary, in Spanish population the same haplotype joins with reduced levels of inflammatory cytokines and lower AD presence [27].

In conclusion, cytokine genetic polymorphisms and their functional expression are very different in various populations of same ethnic origin, and large cohort studies are

necessary in order to assess the true association of some cytokine alleles or haplotypes with the brain damage amplification in AD.

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