SHORT REPORT

A polymorphic variation in the interleukin 1A gene increases brain microglial cell activity in Alzheimer's disease

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Objective: To investigate the impact of possession of the −889 C/T polymorphism of the interleukin 1A gene (IL-1A) and the −511 C/T polymorphism of the interleukin 1B gene (IL-1B) on the extent of neuroinflammation in the brain in Alzheimer's disease (AD), as demonstrated by the degree of microglial cell activity associated with each IL-1A and IL-1B genotype.

Method: Microglial cell activity within the frontal cortex was determined in 68 patients with necropsy confirmed AD by image analysis as the percentage area of tissue occupied by ferritin immunostained material (microglial cell load). IL-1A, IL-1B, and apolipoprotein E (APOE) genotyping were performed by polymerase chain reaction on DNA extracted from frontal cortex or cerebellum.

Results: The microglial cell load was 31% greater in patients with IL-1A T allele, 62% greater with IL-1A TT genotype, but 108% greater with IL-1A TT genotype in combination with APOE ε4 allele. No effects on microglial cell load occurred with polymorphisms in IL-1B, or APOE alone.

Conclusions: Polymorphisms within IL-1A influence the degree of brain microglial cell activation, especially in bearers of APOE ε4 allele, reinforcing the importance of neuroinflammatory processes in the pathogenesis of AD, and supporting the rationale for treating the disease with inflammation modulating drugs.

Alzheimer's disease (AD) is characterised pathologically by the presence of numerous senile plaques and neurofibrillary tangles within the association areas of the neocortex and the archicortical regions of the hippocampus and amygdala. These structural changes are accompanied by a neuroinflammatory response involving the activation of glial cells, both microglial cells and astrocytes, in and around the amyloid β protein (Aβ) component of the senile plaques, and in relation to dead or dying neurofibrillary tangles bearing nerve cells. The production of cytokines or free radicals by these neuroinflammatory cells might damage, and even kill, neurones by triggering or facilitating the production and accumulation of pathological Aβ or tau proteins.[1]

Genetic variations represent major risk factors for AD. Familial early onset AD is associated with mutations in the amyloid precursor protein and presenilin genes, whereas only the polymorphic variation at the apolipoprotein E (APOE) locus, favouring the ε4 allelic form, has so far been firmly established as a genetic risk factor for late onset familial and sporadic AD.[2] However, collectively, these four genetic risk factors only account for about 30% of all cases of AD.[2] Other genetic risk factors might increase the risk of AD by promoting inflammatory reactions, particularly those mediated by the release of interleukin 1 (IL-1) from microglial cells of the brain.[1]

In fact, IL-1 is a family of three related proteins—IL-1A, IL-1B, and the IL-1 receptor antagonist—all of which are encoded within a cluster of genes on chromosome 2. Genetic association studies have sought to implicate polymorphic variations in these proinflammatory genes in the pathogenesis of AD. Several reports have claimed an increased frequency of the −889 C/T promoter polymorphism in the IL-1A gene in AD,[3–7] whereas other work has associated the +3953[6] and −511 C/T promoter[4 7] polymorphisms in IL-1B with an increased risk of AD, especially in conjunction with the −889 IL-1A polymorphism.[6] Both the −889 IL-1A[4] and −511 IL-1B[7 8] polymorphisms have been linked to an earlier age at onset of disease in patients with AD who are homozygous for the rarer T allele, although, paradoxically, a faster rate of decline was seen in patients homozygous for the C allele.[9]

Increased IL-1 expression, in the form of higher tissue concentrations of the IL-1 protein and increased numbers of IL-1 immunoreactive astrocytes, has been demonstrated in the brains of patients with AD and in those elderly individuals with Down's syndrome who show AD-type pathology in their brains.[10] Hence, allelic variations in IL-1 may have a disease modifying role by influencing the degree of glial activation present within the brain tissue, thereby increasing the secretion of putatively damaging cytokines and potentially promoting Alzheimer-type pathological changes. In our present study, we have investigated the impact of polymorphic variations in the IL-1A and IL-1B genes on the degree of neuroinflammation, in terms of the extent of microglial cell activation, in a cohort of patients with pathologically confirmed AD.

PATIENTS AND METHODS

Brains were obtained at necropsy from 68 patients with early and late onset sporadic AD accessioned from the Greater Manchester region of the UK during the years 1986 to 2001 (31 men, 37 women; mean age at onset, 65.9 years; SD, 10.3; range, 40–85). All pathological diagnoses were made in accordance with CERAD neuropathological criteria for AD.[11] Paraffin wax embedded sections (6 μm) were cut from formalin fixed blocks of frontal cortex (BA8/9), and immunostained for ferritin (as a tissue marker of activated microglial cells), using a standard avidin–biotin complex method, as described previously.[12] Briefly, the immunoreaction used a polyclonal antibody to ferritin (Sigma, Poole, Dorset, UK) at a dilution of 1/750. Incubation with the

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; APOE, apolipoprotein E; IL, interleukin

antibody was performed overnight at 4°C; 3,3′ diaminobenzidene was used for visualisation of the antigenic sites. Microglial cell load, expressed as the percentage of tissue section covered by ferritin immunostained material, was measured by image analysis, as described previously.[13] Genomic DNA was extracted from frozen brain tissue by standard methods. The APOE genotype and the IL-1A −889 C/T and IL-1B −511 C/T polymorphisms were determined by polymerase chain reaction.[14–16] Comparisons of microglial cell load between different genotype groups were performed using Kruskal-Wallis non-parametric ANOVA, and when significant, differences between bearers and non-bearers of risk alleles were compared by Mann-Whitney U test.

RESULTS

Table 1 shows the genotype frequencies for the IL-1A, IL-1B, and APOE polymorphisms for this cohort of 68 patients with necropsy confirmed AD. The APOE ε4 allele frequency was 0.38. All allele and genotype frequencies were in Hardy-Weinberg equilibrium. There were no differences in IL-1A (table 2) or IL-1B (data not shown) allele or genotype distribution between bearers and non-bearers of the APOE ε4 allele, and neither did the APOE ε4 allele frequency differ between genotype groups for either IL-1A (CC, 0.40; CT, 0.35; TT, 0.43) or IL-1B (CC, 0.35; CT, 0.41; TT, 0.39).

There were significant differences in mean microglial load between IL-1A genotypes ($p = 0.03$), with an apparent gene dosage effect (table 1). Patients bearing the IL-1A T allele and those with the IL-1A TT genotype had significantly higher microglial loads (by 31% and 62%, respectively) than patients without the T allele ($p < 0.01$). However, there were no significant differences in mean microglial load between the IL-1B genotypes ($p = 0.74$) or between the five APOE genotypes ($p = 0.97$) (table 1). Stratification of IL-1A data in non-bearers of the APOE ε4 allele showed no significant differences in microglial cell load according to IL-1A genotype (table 2). However, microglial cell load was significantly greater ($p < 0.05$) in patients bearing both the APOE ε4 allele and the IL-1A CT (49% higher) or TT (108% higher) genotypes, or the T allele (61% higher) (table 2).

Table 2 IL-1A genotype frequencies and mean (SD) microglial cell loads for 68 patients with Alzheimer's disease, stratified according to possession of the APOE ε4 allele

Gene/Genotype	Genotype frequency	Microglial cell load (% area covered)
IL-1A −889 C/T ε4−	n = 27	
CC	0.48	0.73 (0.63)
CT	0.44	0.72 (0.39)
TT	0.07	0.75 (0.15)
T allele bearers	0.52	0.73 (0.37)
IL-1A −889 C/T ε4+	n = 41	
CC	0.44	0.49 (0.51)
CT	0.44	0.73 (0.47)
TT	0.12	1.02 (0.56)*
T allele bearers	0.56	0.79 (0.50)

*$p<0.05$ compared with CC genotype.

DISCUSSION

We have shown for the first time that the degree of brain inflammation in AD can be affected by variations in proinflammatory genes. Although possession of the IL-1A −889 C/T polymorphism may not necessarily increase the risk of developing AD per se,[16 17] it may play an important role in modifying the course of the disease in sufferers by increasing certain aspects of inflammatory pathology in the brain. Microglial cell activation is greater in the frontal cortex of patients with AD who bear the T allele form of the IL-1A −889 C/T polymorphism, especially in those bearing the TT genotype, and even more so when the APOE ε4 allele is also present. Although the degree of microglial cell activation may vary according to brain area in AD, it is probable that the proportionate changes seen here in the frontal cortex across IL-1A genotypes are mirrored in other brain regions. Such microglial cell changes are specific to the IL-1A −889 C/T polymorphism, and are not seen with the IL-1B −511 C/T polymorphism (vide supra) or the IL-6 −174 C/G promoter polymorphism.[18] Indeed, Nicoll *et al* have suggested that those with compound homozygosity at the IL-1A and IL-1B loci may be at even greater risk of AD.[6] If so, one might expect especially high microglial loads to be present in such patients. Unfortunately, however, we were unable to test this possibility among our present cohort of individuals because there were no patients homozygous for the T allele at both loci included in our study group.

Because the IL-1B −511 C/T polymorphism has been shown to be functionally relevant, with individuals homozygous for the T allele showing a fourfold increase in secreted

Table 1 APOE, IL-1A, and IL-1B genotype frequencies (together with control data) and mean (SD) microglial cell loads for 68 patients with Alzheimer's disease (AD)

Gene/Genotype	Genotype frequency		Microglial cell load (% area covered)
	AD	Control*†	
APOE	n = 68	n = 479	
E2/E3	0.06	0.10	0.58 (0.26)
E2/E4	0.02	0.02	0.38
E3/E3	0.34	0.61	0.75 (0.53)
E3/E4	0.43	0.25	0.68 (0.57)
E4/E4	0.16	0.02	0.63 (0.39)
IL-1A −889 C/T	n = 68	n = 503	
CC	0.44	0.44	0.59 (0.57)
CT	0.46	0.43	0.73 (0.43)
TT	0.10	0.12	0.94 (0.47)‡
T allele bearers	0.56	0.56	0.77 (0.52)‡
IL-1B −511 C/T	n = 68	n = 479	
CC	0.50	0.44	0.61 (0.39)
CT	0.35	0.46	0.77 (0.63)
TT	0.15	0.10	0.85 (0.67)
T allele bearers	0.50	0.56	0.81 (0.63)

*Previously published[16] IL-1 control data from non-demented patients of the Birmingham/Manchester/Scottish UK control cohort is presented for comparison. †Previously unpublished APOE control data from the same Birmingham/Manchester/Scottish UK control cohort; *$p<0.05$ compared with the CC genotype.

concentrations of IL-B,[19] the IL-1A −889 C/T polymorphism may similarly increase tissue expression of IL-1A. The IL-1 protein regulates the expression and processing of amyloid precursor protein.[20] Therefore, overexpression of IL-1 may favour the catabolism of amyloid precursor protein and the formation of Aβ. This, in turn, may promote further microglial activation, especially in the presence of the APOE ϵ4 allele,[21] with further synthesis and release of IL-1 producing feedback amplification, and thereby driving a vicious cycle of cytokine mediated pathology. We have shown elsewhere [16] that the Aβ load is greater in patients with AD bearing the IL-1B −511 T/T genotype but, interestingly, not in those with the IL-1A −889 C/T or T/T genotypes. Such data suggest that the degree of microglial cell activation (reaction) for a given amount of Aβ in the brain is greater in patients with the IL-1A −889 C/T polymorphism than in those without this change, and that the damaging actions of such cells may be mediated through a greater oxidative damage to nerve cells by free radical production, rather than through the promotion of further Aβ deposition. Another aspect of the neuroinflammatory process in AD that may be subject to influence by IL-1A is the degree of astrocytic activation. Increased numbers of IL-1 immunoreactive astrocytes have been shown to be present in AD.[10] Therefore, it is possible that the degree of astrocyte activation may be higher in bearers of the IL-1A T allele, especially those with the IL-1A TT genotype. Studies are in progress investigating this possibility.

Non-steroidal anti-inflammatory drugs are being increasingly used to treat patients with AD, but with variable efficacy.[22] Our data imply such treatment might be most beneficial in that subset of patients, homozygous for the IL-1A polymorphism and bearers of APOE ϵ4 allele, in whom the level of microglial cell activation is greatest. The data also underscore the importance of making genotype/phenotype correlations to evaluate the role and relevance of genetic risk factors in the causation of neurodegenerative disorders such as AD.

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