A Meta-Analysis of Cytokines in Alzheimer’s Disease

Walter Swardfager, Krista Lanctôt, Lana Rothenburg, Amy Wong, Jaclyn Cappell, and Nathan Herrmann

Background: Studies suggest that inflammation is involved in the neurodegenerative cascade leading to Alzheimer’s disease (AD) pathology and symptoms. This study sought to quantitatively summarize the clinical cytokine data.

Methods: Original English language peer-reviewed studies measuring cytokine concentrations in AD and healthy control subjects were included. Mean (± standard deviation) cytokine concentrations for AD and control subjects were extracted.

Results: Forty studies measuring peripheral blood cytokine concentrations and 14 measuring cerebrospinal fluid (CSF) cytokine concentrations were included. In peripheral blood, there were significantly higher concentrations (weighted mean difference [95% confidence interval]) of interleukin (IL)-6 (2.86 [1.68, 4.04] pg/mL, p < .00001, N[AD/control subjects] = 985/680, 14 studies), tumor necrosis factor (TNF)-α (3.25 [1.76, 5.74] pg/mL, p = .01, N = 680/447, 14 studies), IL-1β (5.55 [32, .78] pg/mL, p < .00001, N = 574/370, 10 studies), transforming growth factor (TGF)-β (67.23 [28.62, 105.83] pg/mL, p = .0006, N = 190/158, 5 studies), IL-12 (7.60 [5.58, 9.62] pg/mL, p < .00001, N = 148/106, 5 studies), and IL-18 (15.82 [1.98, 29.66] pg/mL, p = .03, N = 131/94, 4 studies) but not of IL-4, IL-8, IL-10, interferon-γ, or C-reactive protein in AD subjects compared with control subjects. There were significantly higher concentrations of TGF-β (7.81 [2.27, 13.35] pg/mL, p = .006, N = 113/114, 5 studies) but not IL-6, TNF-α, and IL-1β in the CSF of AD subjects compared with control subjects.

Conclusions: These results strengthen the clinical evidence that AD is accompanied by an inflammatory response, particularly higher peripheral concentrations of IL-6, TNF-α, IL-1β, TGF-β, IL-12 and IL-18 and higher CSF concentrations of TGF-β.

Key Words: Alzheimer’s disease, clinical, cytokines, inflammation, interleukin, meta-analysis

Alzheimer’s disease (AD) is the most common form of dementia, with a prevalence of approximately 10% after the age of 65 (1). The complex neurodegenerative cascade leading to AD neuropathology and symptoms is characterized by altered production, aggregation and clearance of the amyloid-β peptide deposited in plaques, and hyperphosphorylation of the tau protein forming neurofibrillary tangles (2–5). Interventions targeted at this cascade to date have been unsuccessful (6,7), while current pharmacotherapy, which is only modestly effective (8–11), does not act on these mechanisms. Therefore, other etiologic hypotheses are needed to help guide the development of alternative or adjunctive treatment strategies with disease-modifying potential.

There is considerable evidence to suggest that an inflammatory response may be involved in the AD neurodegenerative cascade (12,13). For example, pathology studies have shown that the proinflammatory cytokine interleukin (IL)-1β is overexpressed sixfold in the brains of AD patients compared with control subjects (14), especially in the vicinity of amyloid plaques (15). In genetic association studies, a polymorphism in the IL-1β gene has been associated with an increased risk of AD (16) and recent genome-wide association studies have continued to implicate inflammatory pathways. Specifically, a strong independent association has been identified between AD and genetic markers spanning the complement receptor type 1 gene that encodes a regulator of complement activation involved in the phagocytic clearance of immune-pathogen complexes (17,18). Several lines of evidence suggest that complement receptor type 1 may also play a role in the clearance of amyloid-β, suggesting a critical link between proinflammatory immune processes and neurodegeneration (18). However, the clinical implications of the findings from these studies remain unclear (17).

Clinically, associations between AD and many inflammatory biomarkers, including the cytokines IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, interferon (IFN)-γ, tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β, and the acute phase reactant C reactive protein (CRP) have been documented. However, these associations are often inconsistent between studies (19). Moreover, cytokines have been sampled from peripheral blood and from cerebrospinal fluid (CSF), sometimes with discordant results (20). For example, some studies have found higher peripheral blood concentrations of IL-6 in AD patients compared with control subjects, while CSF IL-6 concentrations did not differ between the same AD and control subjects (21). Thus, the clinical literature remains to identify a pattern of immune activation associated with AD.

Results from individual studies can be combined quantitatively using meta-analytical techniques to improve the strength of evidence. Therefore, this study reports the results of a meta-analysis conducted to determine whether the concentrations of specific cytokines differ quantitatively between patients diagnosed with AD and control subjects as measured from peripheral blood and CSF.

Methods and Materials

Data Sources
All analyses were performed according to PRISMA guidelines (22), While PRISMA guidelines have focused on randomized trials, the PRISMA statement specifies “PRISMA can also be used as a basis for reporting systematic reviews of other types of research.” English language literature was searched using Medical Literature Analysis and Retrieval System Online (National Library of Medicine, Bethesda, Maryland), Excerpta Medica Database (Elsevier, Amsterdam, The Netherlands), PsycINFO (American Psychological Association, Washington, DC), Cochrane Library (The Cochrane Collaboration, Oxford, Oxfordshire, United Kingdom), Allied and Complementary Medicine Database (The British Library, London, United Kingdom), and Cumulative Index to Nursing and Allied Health Literature (EBSCO Publishing, Glendale, California) up to December 2009, using the key words Alzheimer’s disease plus cy-
torine, interferon, interleukin, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, TGF-β, IFN-γ, TNF-α, and CRP. Reference lists of relevant studies were searched for additional reports. No standardized review protocol has been published.

Study Selection
Original studies measuring cytokine concentrations in living subjects with AD were included. Inclusion criteria required AD diagnosis based on National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association or DSM-IV (23) criteria or neuropsychological criteria with neuroimaging and inclusion of medically and psychiatrically healthy subjects as control subjects. Studies measuring cytokine concentrations from peripheral blood cells following stimulation were excluded because these assays can introduce variability associated with the immune challenge. Cytokine concentrations from CSF were analyzed separately.

Data Extraction
Three independent raters examined each retrieved article. The results were compared between raters and any disagreements regarding inclusion were settled by consensus. The methods and results sections of each relevant article were analyzed and mean (± SD) cytokine concentrations for AD and control groups were extracted. If data were presented in a format from which means and SDs were not extractable, these measures were requested from the corresponding author of the publication.

Statistical Analyses
A weighted mean difference (WMD) and 95% confidence interval were calculated for each outcome using a random effects model (24). Random effects models are preferable if significant heterogeneity is expected because they assume and account for variable underlying effects in estimates of uncertainty, including both within- and between-studies variances.

A Q statistic was calculated in chi-square analysis to quantify heterogeneity among combined results. A significant Q statistic indicates diversity in the characteristics of the combined trials. Inconsistency was calculated using an I² index to determine the impact of heterogeneity (25). To identify potential sources of heterogeneity, planned subgroup analyses were conducted in studies where control subjects were matched for age, in those excluding subjects with inflammatory comorbidity, and in those using comparable assay methodology, and planned study level meta-regression analyses were conducted comparing WMDs to Mini-Mental Status Examination (MMSE) scores, age, and gender proportion (26). Risk of publication bias was assessed using funnel plots and rank correlation tests between effect size and sample size (27,28). Risk of selective reporting bias was examined within studies. When the difference between groups was not statistically significant, Altman’s method of describing confidence intervals (29) was used. Analyses were conducted using Stata (release 10.1, StataCorp, College Station, Texas).

Results

Literature Search Findings
Search criteria returned 206 unique records of cytokine studies in living subjects with AD (Figure S1 in Supplement 1) and 10 records published in languages other than English. Of the English language records, 98 were excluded based on the publication type being a review rather than a clinical study and 22 were excluded based on reporting only cytokine excretion from peripheral blood following exogenous stimulation. Thus, 86 studies were identified for review.

A total of 73 cross-sectional studies of peripheral blood cytokine concentrations were reviewed. Studies were excluded from meta-analysis based on a lack of healthy control groups (n = 11) (30–40), reporting data in parameters from which WMDs were inestimable (n = 17) (41–56), being a publication reporting on a previously reported population (n = 1) (57), reporting undetectable concentrations of cytokines of interest (n = 3) (58–60), and lack of diagnostic criteria specified for AD (n = 1) (61). Sala et al. (62) and Zuliani et al. (63) provided cytokine means and standard deviations that could not be extracted from their published reports. Thus, 40 studies were included (Table S1 in Supplement 1): 14 for IL-6 (21,62–74), 14 for TNF-α (21,62,63,69,72–81), 10 for IL-1β (63,66,69,71,77,78,82–85), 5 for CRP (66,66–89), 5 for TGF-β (20,90–93), 4 for IL-4 (69,80,94,95), 4 for IL-10 (20,72,74,96), 5 for IL-12 (20,65,92,97,98), 4 for IL-18 (91,92,98,99), 4 for IFN-γ (20,63,65,80), and 3 for IL-8 (74,80,94).

A total of 34 cross-sectional studies of CSF cytokine concentrations were reviewed. Studies were excluded based on a lack of healthy control groups (n = 12) (31,33,37,40,100–107), reporting data in parameters from which WMDs were inestimable (n = 4) (108–111), reporting undetectable concentrations for cytokines of interest (n = 2) (45,59), fewer than three reports of a searched cytokine (n = 1) (112), or being a publication reporting on a previously reported population (n = 1) (113). Rosler et al. (114,115) provided cytokine means and standard deviations that could not be extracted from the published report. Thus, 14 studies satisfied inclusion and exclusion criteria (Table S2 in Supplement 1): 8 for IL-6 (21,60,114,116–120), 5 for TGF-β (20,94,121–123), 3 for TNF-α (21,60,124), and 4 for IL-1β (21,60,118,119).

Cytokine Concentrations
Significantly higher peripheral blood cytokine concentrations were detected in AD subjects compared with healthy control subjects in comparisons of IL-6, TNF-α, IL-1β, TGF-β, IL-12, and IL-18 (Figures 1–6, Table 1). Weighted mean differences were not significant for the other cytokines investigated. A significantly higher WMD in CSF cytokine concentrations of TGF-β was detected in AD subjects compared with healthy control subjects (Figure 7, Table 2).

Investigation of Heterogeneity
All cytokine concentrations were compared in picograms per milliliter; however, significant heterogeneity was found in most comparisons (Figures 1–7, Tables 1 and 2).

The main analyses were repeated in a subgroup of 21 studies specifically matching control subjects for age (Table S1 in Supplement 1). For IL-1β (Figure 3), the impact of heterogeneity was slightly reduced but the significance of the association between elevated plasma IL-1β concentrations and AD was lost (p = .26, I² = 87%, N = 119/71 in 4 studies). The impact of heterogeneity was slightly reduced in the IL-6 comparison, the significance of which was retained (p = .02, I² = 86%, N = 214/153 in 5 studies). Studies measuring IL-4, IL-8 and IFN-γ numbered less than 3, so they could no longer be combined. The significance of the results and the impact of heterogeneity did not differ from those of the main analyses for TNF-α, CRP, TGF-β, IL-10, and IL-12. Of 16 studies undertaking CSF cytokine measurement, 7 studies matched control subjects for age; the IL-6 comparison remained insignificant and studies of TGF-β, TNF-α, and IL-1β numbered less than 3 so they could no longer be combined. In meta-regression analyses, the mean age of the AD group was not significantly associated with the WMD for any of the cytokines examined in peripheral blood or CSF.
Only 3 peripheral blood studies and 2 CSF studies matched control subjects for gender, precluding subgroup analyses. In meta-regression analyses, no associations between the WMD and the proportion of AD subjects who were male could be detected for any of the cytokines examined in peripheral blood or CSF.

Mean MMSE scores were reported in 7 studies measuring peripheral blood IL-1β (Table S1 in Supplement 1), 4 measuring peripheral blood TNF-α (Table S1 in Supplement 1), and 6 measuring CSF IL-6 (Table S2 in Supplement 1). Meta-regression analyses were carried out to assess the association of each WMD with the mean MMSE score of the AD group as an indicator of disease severity. The WMD between AD and control subjects in peripheral blood IL-1β was correlated with mean MMSE scores (coefficient = .752, 95% confidence interval: .44 –1.06, p = .002; Figure S2 in Supplement 1). No associations between WMD and MMSE scores could be detected for any of the other cytokines examined in peripheral blood or CSF.

Twenty-six studies of peripheral blood cytokine concentrations excluded patients with any inflammatory medical comorbidity.

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**Figure 1.** Studies of peripheral blood interleukin-6. Forest plot displaying random-effects meta-analysis results of the association between peripheral blood interleukin-6 concentrations and Alzheimer’s disease. Weighted mean difference (picograms per milliliter) on horizontal axis; positive values denote higher in Alzheimer’s disease patients; negative values denote higher in healthy control subjects. Hashed line at the overall weighted mean difference. CI, confidence interval; WMD, weighted mean difference.

**Figure 2.** Studies of peripheral blood tumor necrosis factor-α. Forest plot displaying random-effects meta-analysis results of the association between peripheral blood tumor necrosis factor-α concentrations and Alzheimer’s disease. Results are separated into subgroups of studies that excluded inflammatory medical comorbidity and those that did not. Weighted mean difference (picograms per milliliter) on horizontal axis; positive values denote higher in Alzheimer’s disease patients; negative values denote higher in healthy control subjects. Hashed line at the overall weighted mean difference. CI, confidence interval; WMD, weighted mean difference.
In subgroup analyses of these studies, neither the significance of the main effect nor the impact of heterogeneity differed from those in the main analyses except for the TNF-\(\beta\)/H9251 comparison (Figure 2), the significance of which was lost \(p = .67, I^2 = 78\%\), \(N = 361/166\) in 8 studies). Among CSF studies, only 6 excluded subjects with inflammatory conditions (IL-1 was measured in 1 study, IL-6 in 3 studies, TNF-\(\beta\) in 3 studies, and TGF-\(\beta\) in 2 studies). Comparisons for both IL-6 and TNF-\(\beta\) remained nonsignificant and the impact of heterogeneity did not differ from those of the main analyses.

For determinations of peripheral blood IL-6, TNF-\(\beta\), IL-1\(\beta\), IL-12, and IL-18, enzyme-linked immunosorbent assay (ELISA) kits from 4, 10, 5, 3, and 4 different suppliers were used, respectively. The majority of studies used noncompetitive sandwich ELISA techniques, while some used competitive or reverse ELISA techniques or bioassays. In subgroup analyses including only studies that used sandwich ELISA assays, the impact of heterogeneity on comparisons of peripheral blood TNF-\(\beta\) \(N = 501/288\) in 10 studies) and TGF-\(\beta\) \(N = 224/209\) in 5 studies) was slightly reduced \(I^2 = 93\%\) and \(I^2 = 92\%\), respectively); however, the associations between AD and peripheral blood concentrations were nonsignificant for both cytokines \(p = .91\) and \(p = .08\), respectively). Only 2 studies were available for IL-8 in this subgroup analysis such that a comparison could not be made and CRP was not assayed by ELISA. Other comparisons did not differ from the main analyses in the significance of the effect or in the impact of heterogeneity. Intra-assay and interassay coefficients of variation, assay sensitivity, and the proportion of undetectable samples were not consistently reported.

**Publication and Selective Reporting Bias**

Significant risk of publication bias was not detected as demonstrated by funnel plots and no significant correlations between effect size and sample size among studies of peripheral blood IL-6 \(p = -.495, p = .085\); TNF-\(\beta\) \(p = -.115, p = .707\); IL-1\(\beta\) \(p = -.336, p = .342\); TGF-\(\beta\) \(p = .400, p = .505\); IL-12 \(p = .800, p = .200\); and IL-18 \(p = .600, p = .400\) concentrations or among studies of TGF-\(\beta\) CSF concentrations \(p = -.300, p = .624\).

Simultaneous reporting of negative results suggested a lower risk of reporting bias; the majority of included studies examined multiple biomarkers and the majority of studies reporting at least one significant comparison also reported at least one nonsignificant comparison. Reports of only significant findings were published before the implementation of multiplex assays \(75,90\), by authors \(76,83,84\) who also published contemporaneous negative
findings in other included reports (66,82,87), or in a chiefly genetic study (81).

Discussion

This meta-analysis reports significantly higher concentrations of the proinflammatory cytokines IL-6, TNF-α, IL-1β, IL-12, and IL-18 in the peripheral blood of AD subjects compared with control subjects. Evidence was particularly strong for IL-6, IL-12, and IL-18, which were significantly associated with AD in subgroups of studies that matched control subjects for age, in which subjects were free of inflammatory comorbidity and that used comparable assay techniques. While both positive and negative results have been reported in individual studies, these meta-analytic results strengthen the clinical evidence that AD is accompanied by a peripheral inflammatory response. This meta-analysis did not find support for the involvement of the other proinflammatory cytokines IL-8 and IFN-γ, though fewer studies assessed concentrations of these cytokines resulting in smaller population sizes, which may have made it more difficult to observe associations.

Concentrations of IL-4 and IL-10 did not differ significantly between subjects with AD and control subjects. Functionally, IL-4 and IL-10 oppose the actions of the proinflammatory cytokines IL-6 by stimulating the differentiation of naive T cells into noninflammatory T cell types and IL-10 by suppressing cytokine production from all T cell types (125–130). Though these findings cannot be interpreted as truly negative due to smaller sample sizes and significant heterogeneity between studies, a lack of measurable compensatory increase in anti-inflammatory signaling might further imply a proinflammatory immune imbalance. This meta-analysis did, however, confirm higher plasma concentrations of TGF-β in AD compared with control subjects, for which anti-inflammatory properties have been described (130,131). However, the role of TGF-β remains controversial in AD, particularly because naive T cells stimulated with TGF-β may take on a proinflammatory phenotype in the presence of IL-6 (132), which this meta-analysis also found to be consistently elevated in subjects with AD. In mouse models, blockade of peripheral TGF-β signaling has been found to ameliorate AD pathology, suggesting that the presence of TGF-β may too be deleterious (133).

The clinical significance of cytokine elevations remains a subject of debate and peripheral measurements are limited in that they may not specifically reflect inflammatory activity within the central nervous system (CNS). While smaller sample sizes and significant heterogeneity are noted, with the exception of TGF-β this meta-analysis did not find significant evidence supporting increased concentrations of the examined cytokines in the CSF of AD subjects. With evidence reviewed elsewhere (12-15,19,150) demonstrating inflammatory activity within the AD brain the possible pathophysiological significance of peripheral proinflammatory cytokines could be considered (151).

Mechanisms have been described whereby a peripheral inflammatory response can contribute to neurotoxicity that does not depend directly on cytokine secretion from within the CNS, for example, by increasing amyloid-β deposition in the brain. In a murine model, peripheral administration of lipopolysaccharide (LPS) increased influx and decreased efflux of amyloid-β across the blood brain barrier, while also increasing neuronal synthesis of amyloid-β (134). Increased amyloid-β influx was not due to LPS directly, but rather it correlated with circulating IL-6 levels. Pretreatment with the nonsteroidal antiinflammatory drug indomethacin corrected the impaired efflux of amyloid-β induced by LPS, suggesting the possible benefit of certain anti-inflammatory agents in AD. In human neuronal cell cultures, pretreatment with ibuprofen decreased amyloid-β secretion by 50% and prevented its accumulation when the neurons were stimulated with IFN-γ or TNF-α (135).

Peripheral proinflammatory signals can be actively propagated across the blood brain barrier by crosstalk between peripheral and central immune cells (19,136–139). Cytokines produced peripher-

### Table 1: Studies of peripheral blood interleukin-12 concentrations and Alzheimer's disease

<table>
<thead>
<tr>
<th>Study</th>
<th>WMD (95% CI), pg/ml</th>
<th>Weight, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh et al., 1997 (65)</td>
<td>3.57 (1.09, 8.23)</td>
<td>13.44</td>
</tr>
<tr>
<td>Rota et al., 2006 (20)</td>
<td>-1.50 (-21.93, 18.93)</td>
<td>0.82</td>
</tr>
<tr>
<td>Guerreiro et al., 2007 (97)</td>
<td>8.89 (3.83, 13.95)</td>
<td>11.68</td>
</tr>
<tr>
<td>Motta et al., 2007 (92)</td>
<td>8.22 (7.43, 9.01)</td>
<td>74.05</td>
</tr>
<tr>
<td>Lee et al., 2008 (98)</td>
<td>-5.80 (-163.45, 151.85)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Overall total</strong></td>
<td>7.60 (5.58, 9.62)</td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

Heterogeneity ($I^2=14.5, p=.322$)

### Figure 5: Studies of peripheral blood interleukin-12 concentrations and Alzheimer's disease. Weighted mean difference (picograms per milliliter) on horizontal axis; positive values denote higher in Alzheimer's disease patients; negative values denote higher in healthy control subjects. Hashed line at the overall weighted mean difference. CI, confidence interval; WMD, weighted mean difference.

### Table 2: Studies of peripheral blood interleukin-18 concentrations and Alzheimer's disease

<table>
<thead>
<tr>
<th>Study</th>
<th>WMD (95% CI), pg/ml</th>
<th>Weight, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaguemera et al., 2006 (91)</td>
<td>34.95 (26.13, 41.77)</td>
<td>30.32</td>
</tr>
<tr>
<td>Motta et al., 2007 (92)</td>
<td>16.11 (14.40, 17.82)</td>
<td>34.28</td>
</tr>
<tr>
<td>Bossi et al., 2008 (99)</td>
<td>-5.80 (46.21, 34.61)</td>
<td>8.75</td>
</tr>
<tr>
<td>Lee et al., 2008 (98)</td>
<td>0.90 (-11.77, 13.57)</td>
<td>26.74</td>
</tr>
<tr>
<td><strong>Overall total</strong></td>
<td>15.82 (1.98, 29.66)</td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

Heterogeneity ($I^2=87.5, p=.000$)

### Figure 6: Studies of peripheral blood interleukin-18 concentrations and Alzheimer's disease. Weighted mean difference (picograms per milliliter) on horizontal axis; positive values denote higher in Alzheimer's disease patients; negative values denote higher in healthy control subjects. Hashed line at the overall weighted mean difference. CI, confidence interval; WMD, weighted mean difference.

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ally can cross the blood brain barrier by saturable transporters (140–144) or by passive diffusion through spaces between vascular endothelial cells (145,146). Also, activated peripheral immune cells can migrate across the blood brain barrier, in response to chemokine receptor signaling (139,147). In the short term, infiltrating cells can migrate across the blood brain barrier, in response to chemokine receptor signaling (139,147). In the short term, infiltrating cells may reduce disease burden by clearing amyloid-β (139); however, in AD, the microglia are especially reactive to inflammatory stimuli, possibly primed by exposure to amyloid-β plaques and dysregulated complement activation (17,18), to secrete inflammatory mediators such as IL-1β and TNF-α inside the CNS (19,148–150). Therefore, over time, it has been suggested that the net effect of microglial activation might be to further increase amyloid-β deposition, perpetuating the neurodegenerative cascade (19,151).

Another candidate mechanism relating peripheral proinflammatory cytokines to neurodegeneration is their capacity to induce indoleamine 2,3-dioxygenase (IDO), which catalyzes the rate-limiting step in the synthesis of kynurenine from tryptophan (152–154). Peripheral kynurenine synthesis contributes substantially to CNS concentrations (155–157), and an increased concentration of quinolinic acid, a neurotoxic kynurenine metabolite, has been observed in the hippocampus of AD subjects compared with control subjects, concentrated around plaques and tangles (158,159). The pathological significance of quinolinic acid in AD is suggested by its capacity to induce tau hyperphosphorylation (158,159). Although clinical studies have been limited, Widner et al. (160) observed that IDO activity was significantly increased in AD patients and that the extent of IDO activation correlated with peripheral inflammatory markers and poorer cognitive performance (160).

In this meta-analysis, the possibility that cytokine elevations may arise transiently, as in acute infection, or continuously because of chronic disease may have contributed to heterogeneity, and both cases may be relevant to AD. For example, acute systemic infections of any kind over 4 years increased the likelihood of incident dementia in a case-control study of the very old (161). Dementia has also been associated with periodontitis and with the corollary increases in peripheral concentrations of TNF-α and antibody titers against periodontal bacteria (162,163). Acute systemic infections have also been associated with increased rates of disease progression, an effect related to elevations in peripheral concentrations of TNF-α and IL-1β (164,165). Among chronic inflammatory conditions, there is considerable evidence linking AD with cardiovascular disease (161,166–172). For example, the density of AD brain lesions has been associated with the extent of coronary artery disease or cerebrovascular changes in histopathological studies, especially in carriers of the ApoE4 allele (172,173). In a longitudinal study, vascular factors predicted a greater rate of decline on the MMSE over 3 years in subjects with incident AD (171). Cardiovascular disease is associated with chronically elevated plasma concentrations of IL-1β, IL-6, TNF-α, and IL-18 (174–181), and IL-18 in particular has been linked with vascular cognitive impairment (182–184). Although many studies included in this meta-analysis excluded subjects with a history of stroke or acute coronary events, these conditions were not consistently reported, and the presence of cardiovascular or cerebrovascular disease cannot be ruled out as factors contributing to cytokine elevations in AD (185).

The direction of causation cannot be inferred from observational studies; thus, the possibility that increased concentrations of proinflammatory cytokines may be a consequence of AD pathology should also be considered. In one large prospective study, elevations in plasma IL-6 concentrations were associated with the future

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**Table 1. Summary of Comparative Outcomes for Peripheral Blood Cytokine Measurements**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Studies</th>
<th>N (AD/NC)</th>
<th>WMD</th>
<th>95% CI</th>
<th>Z</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>IL-6</td>
<td>14</td>
<td>985/680</td>
<td>2.86</td>
<td>1.68, 4.04</td>
<td>4.76</td>
<td>&lt;.00001</td>
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<tr>
<td>TNF-α</td>
<td>14</td>
<td>680/447</td>
<td>3.25</td>
<td>.76, 5.74</td>
<td>2.55</td>
<td>.01</td>
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<tr>
<td>IL-1β</td>
<td>10</td>
<td>574/370</td>
<td>.55</td>
<td>.32, .78</td>
<td>4.72</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>CRP</td>
<td>5</td>
<td>253/155</td>
<td>−.32</td>
<td>−1.80, 1.16</td>
<td>.42</td>
<td>.67</td>
</tr>
<tr>
<td>TGF-β</td>
<td>5</td>
<td>190/158</td>
<td>67.23</td>
<td>28.62, 105.83</td>
<td>3.41</td>
<td>.0006</td>
</tr>
<tr>
<td>IL-10</td>
<td>4</td>
<td>156/123</td>
<td>1.21</td>
<td>−17.33, 19.74</td>
<td>.13</td>
<td>.90</td>
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<tr>
<td>IL-12</td>
<td>5</td>
<td>148/106</td>
<td>7.60</td>
<td>5.58, 9.62</td>
<td>7.99</td>
<td>&lt;.00001</td>
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<td>1.98, 29.66</td>
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<td>.03</td>
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<td>IFN-γ</td>
<td>4</td>
<td>113/102</td>
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<td>−.32, .65</td>
<td>.65</td>
<td>.55</td>
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<td>68/66</td>
<td>−.42</td>
<td>−1.94, −1.10</td>
<td>.54</td>
<td>.59</td>
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</table>

CI, confidence interval; AD, Alzheimer’s disease; CRP, C reactive protein; IFN, interferon; IL, interleukin; NC, normal control; TGF, transforming growth factor; TNF, tumor necrosis factor; WMD, weighted mean difference.

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**Figure 7.** Studies of cerebrospinal fluid transforming growth factor-β. Forest plot displaying random-effects meta-analysis results of the association between cerebrospinal fluid transforming growth factor-β concentrations and Alzheimer’s disease. Weighted mean difference (picograms per milliliter) on horizontal axis; positive values denote higher in Alzheimer’s disease patients; negative values denote higher in healthy control subjects. Hashed line at the overall weighted mean difference. CI, confidence interval; WMD, weighted mean difference.
onset of AD, suggesting that inflammatory activation may be a predisposing or precipitating factor (186). However, the finding that elevations in plasma IL-6 concentrations precede the onset of clinical AD symptoms does not preclude the presence of AD neuropathology at the time of baseline assessment (186). It has been shown that vagus efferents can activate nicotinic acetylcholine receptors containing the α7 subunit on peripheral macrophages, limiting their release of proinflammatory cytokines (187). Activation of this cholinergic anti-inflammatory pathway can be increased by central cholinergic neurotransmission and reduced by brain acetylcholinesterase activity (188). For instance, observations from murine models suggest that brain cholinesterase activity plays a role in regulating the release of TNF-α from peripheral tissues through this pathway and cholinesterase inhibitors can reduce peripheral IL-1β, IL-6, and TNF-α concentrations (69,188,189). If the brain cholinergic neurons comprising the regulatory circuitry of the cholinergic anti-inflammatory pathway were compromised, increased peripheral cytokine secretion could result. This and other in vitro evidence suggest that variations in the use of cholinesterase inhibitors between studies in the present meta-analysis may have contributed to the observed heterogeneity in comparisons of cytokine concentrations (190).

In addition to clinical confounders, variability in assay procedures may have contributed substantially to heterogeneity. Even in a subgroup of studies employing noncompetitive ELISA methodology, inconsistency was identified. In a study of uncertainty among cytokine determinations, Noble et al. (191) submitted a standardized cytokine preparation to 11 specialized laboratories, which returned values varying between 67% and 136% of the standardized mean value. In that report, interplate variability ranged from 5% to 30%, suggesting that a relatively large sample size might be required to obtain significant results in any particular laboratory. Between laboratories, assay performance parameters such as signal-to-noise ratio and quantitative range showed considerable variability within the range of expected clinical cytokine values. This may be particularly problematic with concentrations near the limit of detectability, which is frequently the case, for example, in IL-1β determinations (66). Appreciable heterogeneity is therefore expected when combining assay results from different laboratories.

It has been suggested that studies of large populations employing multiplex assays might be required to adequately describe changes in the cytokine network associated with AD (192), particularly because, as discussed, many of the cytokines investigated can modify the activities of others or act additively on downstream signaling pathways (150). More sophisticated analyses that take into account these interactions at the subject level and integrate them with concentrations of CSF markers such as amyloid-β, total tau and phosphorylated tau might be informative (117,193). In addition, the present meta-analysis identified differences in mean MMSE scores as a potential source of heterogeneity in the IL-1β comparison, showing stronger effect sizes in studies of subjects with milder symptoms. This observation may reflect findings from prospective studies and studies across multiple stages, which suggest that separation of certain cytokines can vary with AD progression (45,62,73,92,192). Longitudinal cognitive assessment with repeated cytokine measurements might better characterize these changes. The relationship between cognitive response to pharmacotherapy and changes in inflammatory biomarkers has not been qualified. The effects of preventative or aggressive treatment against common infections (194) or of investigational immunomodulatory therapies (133,195,196) on inflammatory biomarkers might also be explored in relation to clinical outcomes (197). In addition, effects of vascular risk factor control on cytokine concentrations in AD might be informative because peroxisome proliferator-activated receptor gamma agonists (198,199), statins (200–202), antihypertensive medications (171,202,203) and cardio-pulmonary fitness (204,205) have been associated with reduced inflammatory activation and slower progression of AD.

The present meta-analysis was limited at the level of the literature search to unstimulated cytokines and CRP; however, other inflammatory markers (e.g., chemokines, soluble cytokine receptors, complement proteins, etc.) have also been informative (111,112,120,150,160). This meta-analysis was also limited by substantial inconsistency in most comparisons, necessitating the use of random effects models that produce wider confidence intervals. Most studies reported large standard deviations, suggesting substantial interindividual variation in cytokine concentrations that was not explained. Some potential sources of heterogeneity, including assay methodology, age, gender, and medical comorbidity, were investigated. Although the associations between AD and proinflammatory cytokines, with the exception of TNF-α, persisted in subgroups without inflammatory comorbidity, this does not strictly preclude the use of anti-inflammatory agents in any group, which was not reported consistently. In addition, the use of cholinesterase inhibitors was not reported consistently. The present study was also limited by a categorical diagnosis of AD, and thus AD severity effects could not be systematically investigated. Meta-regression analyses in subgroups of studies reporting mean MMSE scores suggested that differences in AD severity might have contributed to heterogeneity. Furthermore, in cross-sectional studies, continuous and stochastic elevations cannot be distinguished and differences in the secretion patterns and half-lives of different cytokines may have considerable impact on their measured concentrations (206). Differences in ELISA assay techniques were investigated as a potential source of heterogeneity; however, many other assay parameters and methodological differences in the collection and handling of the samples that could not be systematically addressed may have contributed. Funnel plots and Spearman correlations did not suggest the presence of publication bias; however, many observational studies were not registered with clinical trials databases, so the scope of the unpublished literature cannot be ascertained and effects of bias at the study and outcome levels cannot be

### Table 2. Summary of Comparative Outcomes for CSF Cytokine Measurements

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Studies</th>
<th>N (AD/NC)</th>
<th>WMD</th>
<th>95% CI</th>
<th>Z</th>
<th>p</th>
<th>Chi²</th>
<th>df</th>
<th>p</th>
<th>I²</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>8</td>
<td>193/153</td>
<td>.30</td>
<td>–.63, 1.22</td>
<td>.64</td>
<td>.53</td>
<td>32.78</td>
<td>7</td>
<td>&lt;.00001</td>
<td>79%</td>
</tr>
<tr>
<td>TGF-β</td>
<td>5</td>
<td>113/114</td>
<td>7.81</td>
<td>2.27, 13.35</td>
<td>2.76</td>
<td>.006</td>
<td>39.18</td>
<td>4</td>
<td>&lt;.00001</td>
<td>93%</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3</td>
<td>106/71</td>
<td>14.56</td>
<td>–17.16, 46.28</td>
<td>.09</td>
<td>.37</td>
<td>101.38</td>
<td>2</td>
<td>&lt;.00001</td>
<td>98%</td>
</tr>
<tr>
<td>IL-1β</td>
<td>4</td>
<td>84/73</td>
<td>–.86</td>
<td>–3.72, 1.99</td>
<td>.59</td>
<td>.55</td>
<td>39.18</td>
<td>3</td>
<td>&lt;.00001</td>
<td>92%</td>
</tr>
</tbody>
</table>

CI, confidence interval; AD, Alzheimer’s disease; CSF, cerebrospinal fluid; IL, interleukin; NC, normal control; TGF, transforming growth factor; TNF, tumor necrosis factor; WMD, weighted mean difference.

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