Research Article

Altered Serum and Cerebrospinal Fluid Inflammatory Cascades in Mild Cognitive Impairment and Alzheimer's Disease

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Abstract

Paired serum and cerebrospinal fluid (CSF) samples from 21 controls, 8 subjects with mild cognitive impairment (MCI), and 10 with Alzheimer's disease (AD) were analyzed for inflammatory cytokine/chemokine and related trophic factor expression using a multiplex ELISA. Since results obtained from the MCI and AD samples were similar, those data were combined (MCI/AD) to simplify the analysis. In MCI/AD serum samples, the mean levels of IL-1β, IL-4, IL-5, TNF-α, RANTES, IL-13, IL-17A, MIP-1α, eotaxin and PDGF-BB were significantly elevated relative to control, whereas IL-15, IP-10, MCP-1, and GMCSF were reduced. In MCI/AD CSF, IL-5 and IL-13 were significantly elevated whereas GM-CSF, IL-17A, b-FGF, PDGF-BB, and VEGF were reduced. The findings suggest that in the early stages of neurodegeneration, inflammatory factors driving cognitive impairment may be derived more from systemic than CNS responses. In contrast, alterations in trophic factor expression that would adversely affect neuronal survival, neuroprotection, angiogenesis, and myelin integrity may largely originate within the CNS, although they could be propagated by neuroinflammation.

Keywords: Alzheimer's disease, cerebrospinal fluid, serum, cytokines, chemokines, trophic factors, mild cognitive impairment, neuro-inflammation

Introduction

Alzheimer's disease (AD) is characterized by progressive behavioral changes, loss of recent or short-term memory, and declines in executive function and other cognitive abilities [1]. Typically, neurodegeneration begins in a pre-symptomatic stage [2] that may offer the best opportunity to reverse disease or substantially retard its

progression. The histopathological hallmarks of AD include co-accumulations of structural lesions mediated by abnormal hyper-phosphorylation of tau and excessive and aberrant cleavage of the amyloid-beta precursor protein ($A\beta_{1-42}$), yielding phospho-tau immunoreactive fibrillar deposits in neurofibrillary tangles, dystrophic neurites and neuropil threads, and $A\beta_{1-42}$ deposits in plaques and blood vessels. Under normal circumstances, $A\beta_{1-42}$, a ~4 kD peptide generated by secretase cleavage of amyloid precursor protein, is continuously cleared via transport from the brain to the general circulation [3]. However, in AD, $A\beta_{1-42}$ accumulates as fibrillar aggregates in cortical and leptomeningeal blood vessels, perivascular spaces, and plaques, and as neurotoxic, oligomeric diffusible ligands (ADDLs) [4,5]. Cellular stress related to both pTau and $A\beta_{1-42}$ accumulations leads to ubiquitination of their insoluble fibrillar aggregates [6-8], followed by activation of the unfolded protein response (UPR), loss of neuronal function, and ultimately cell death. These abnormalities increase in the brain with progression of neurodegeneration [9].

Diagnostic criteria for rendering a clinical diagnosis of AD has been aided by positron emission tomography (PET) neuroimaging to detect accumulations of pTau and $A\beta_{1-42}$ using F18 isotopically-labeled tracers [10,11], and CSF biomarker panels that include measurements of pTau and $A\beta_{1-42}$ [12-14]. In addition, postmortem neuropathological assessments have been streamlined, assigning grades of AD severity based on abundances of neurofibrillary tangles and senile plaques in particular brain regions [7]. However, in reality, this narrow approach falls short because the nature and distribution of neurodegeneration are far broader and both pTau and $A\beta_{1-42}$ accumulations occur in other disease processes including other forms of neurodegeneration, traumatic brain injury, and normal aging. Thus, an emerging conceptual approach is to incorporate multimodal pathogenic factors to better understand the nature of disease and expand therapeutic targets [15]. One major AD-associated abnormality that is not been well understood but amenable to treatment is neuro-inflammation [16].

Neuro-inflammation is mediated by microglial cell and astrocyte elaboration of pro-inflammatory cytokines, chemokines, complement, and reactive oxygen and reactive nitrogen species [17-19]. Postmortem findings of increased microglial and reactive astrocyte expression of pro-inflammatory cytokines such as IL-1β, IL-6, interferon-gamma (IFN-γ), and macrophage migration inhibitory factor near $A\beta_{1-42}$ plaques suggest that neuro-inflammation may be linked to $A\beta_{1-42}$ deposition [20,21]. Neuro-inflammation causes injury to neurons, impairs cholinergic function, and activates stress signaling pathways [22] leading to increased levels of reactive oxygen and nitrogen species. Attendant damage to nerve terminals disrupts synaptic connections and causes cognitive impairment [18]. Thus, it is conceivable that neuro-inflammatory responses can mediate transitions from normal aging to mild cognitive impairment (MCI) and eventually AD.

Systemic inflammatory responses manifested by elevated serum levels of pro-inflammatory cytokines have been well documented in AD [23]. Furthermore, it has been established that cytokines can cross the blood-brain barrier, possibly via saturable transport mechanisms [24]. Therefore, although neuro-inflammatory responses are mediated by local activation of cytokines and chemokines in brain microglia and astrocytes and cerebrovascular endothelial cells, potential contributions of co-occurring systemic inflammatory responses should be investigated. Importantly, peripheral or systemic inflammation that is capable of driving or contributing to neuro-inflammation, or develops as a secondary component (phase) of neuro-inflammation, should be detectable in peripheral blood. On the other hand, if the major sources of neuro-inflammation are intrinsic and selectively involve the brain, then peripheral responses would not be expected to mirror inflammatory profiles in the brain. These points are important because diagnostic and therapeutic approaches to neuro-inflammatory mediators of neurodegeneration may be informed by the onset, nature, and progression of systemic inflammatory responses. However, without such information, the timing and nature of neuroprotective anti-inflammatory and anti-oxidant treatments may be too late or inadequate.

This point especially resonates with failed clinical trials of anti-inflammatory and anti-oxidant treatments designed to remediate cognitive impairment and neurodegeneration [25].

The present study uses paired serum and cerebrospinal fluid (CSF) samples to compare systemic and central nervous system (CNS) inflammatory responses in clinically well characterized patients with MCI or early stage AD. The goals were to gauge the degree to which systemic inflammation predicts or correspond with neuro-inflammation, and determine if MCI and AD were distinguishable from normal controls based on neuro-inflammatory or systemic inflammatory profiles.

Methods

Human Subjects

The Lifespan Hospitals Institutional Review Board (IRB) approved this study. This cross-sectional study was designed to evaluate inflammatory profiles in prospectively banked paired serum and CSF samples from patients with MCI or AD. The patients were evaluated at the Rhode Island Hospital Alzheimer's Disease and Memory Disorders Center between 2010 and 2016, and the biological fluid samples were collected in accordance with the Alzheimer's Disease Neuroimaging Initiative (ADNI) protocol. An AD diagnosis was rendered based on NINCDS/ADRDA criteria [1,26], and MCI was diagnosed using consensus criteria [27]. Paired serum and lumbar puncture CSF samples were obtained as part of a neurologic diagnostic evaluation or as add-on donations at the time of a clinical trial or observational research study visit. All subjects signed consent forms approved by the Rhode Island Hospital IRB to have their serum and CSF samples banked for future research.

Control patients were evaluated for headache in the Rhode Island Hospital, Miriam Hospital, or Newport Hospital Emergency Department between October 2014 and December 2015. Inclusion criteria for control subjects were that: 1) they were at least 21 years of age and cognitively normal by standard neurological exam and review of the hospital record; 2) paired blood and CSF samples were collected in accordance with standard-of-care hospital practice; 3) their diagnostic studies including CSF protein, cell counts, glucose, and Gram stain were negative; and 4) their emergency room courses were uneventful and ended in discharge to home. An additional eligibility requirement for both groups was that at least 500 µl of the paired undiluted serum and CSF samples were available for these studies. Although the controls were not specifically screened for active or underlying inflammatory disease processes, the clinical records and routine assays of serum and CSF provided no evidence to support this potential confounder. Following collection, the paired samples were aliquoted into 1 mL sterile polypropylene screw capped tubes and stored frozen at -80°C. All samples were hemoglobin-free and prior to use they were filtered (0.45 µM pore) to eliminate cellular debris.

Direct Binding Enzyme-linked Immunosorbent assay (ELISA)

CSF and serum $A\beta_{1-42}$ and phospho-tau (pTau-307) levels of immunoreactivity were measured using direct binding ELISAs [28]. These assays were not performed for diagnostic purposes, but rather for research comparisons of their relative levels with respect to severity of cognitive impairment. Serum samples diluted 1:100 and CSF diluted 1:4 in Tris-buffered saline (TBS) were adsorbed (50 µl each) to the well bottoms of Nunc Maxisorp 96-well plates (Thermo Fisher Scientific Inc., East Providence, RI) by overnight incubation at 4°C, then blocked for 3 hours at room temperature with 1% bovine serum albumin (BSA) in TBS. After washing, the samples were incubated with primary antibody (0.1-0.4 µg/ml) for 1 hour at 37°C. Immunoreactivity was detected with horseradish peroxidase-conjugated secondary antibody and Amplex UltraRed soluble fluorophore. Fluorescence intensity was measured (Ex 565 nm/Em 595 nm) in a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA).

Multiplex Human Cytokine ELISA

Bead-based multiplex ELISAs were employed to assess levels of 27 pro-inflammatory cytokines and chemokines in serum and CSF using the Bio-Plex Pro™ Human Cytokine 27-plex Assay (Bio-Rad, Hercules, CA). The list of cytokines, chemokines, and trophic factors, their abbreviations, and both systemic and CNS functions are summarized in Table 1. Following the manufacturer's protocol, serum diluted 1:4 in assay dilution buffer, and undiluted CSF samples were incubated with magnetic beads that were covalently coupled with capture antibodies. Captured antigens were detected with biotinylated secondary antibodies followed by a streptavidin-phycoerythrin reporter conjugate. Fluorescence intensity was measured in a MAGPIX (Bio-Rad, Hercules, CA) and cytokine/chemokine concentrations (pg/mL) were software-generated (Bio-Rad, Hercules, CA) from standard curves.

Table 1: Cytokine/Chemokine Systemic Functions and Roles in Neurodegeneration

Cytokine/ Chemokine	Full -Other Names	Systemic Functions	Roles in Neurodegeneration	Citations [29-36]
b-FGF	Basic fibroblast growth factor; FGF2	Angiogenic and broad-spectrum mitogenic factor; localized in basement membranes and vascular subendothelial extracellular matrix; cytoprotective; role in wound healing	Both a neurotrophin and mediator of neuronal injury; signals through pathways leading to neurogenesis (dentate gyrus of hippocampus) and neurodegeneration, e.g. following traumatic brain injury. Increased levels in AD and other forms of neurodegeneration. Immunoreactivity increased in astrocytes and with senile plaques, neurofibrillary tangles, and neuropil threads.	[29,37]
Eotaxin	Eosinophil chemotactic protein; CCL11 (eotaxin-1), CCL24 (eotaxin-2), and CCL26 (eotaxin-3)	CC Chemokine subfamily of proteins chemotactic for eosinophils; binds to CCR2, CCR3, CCR5; high levels associated with aging; current cannabis use, and schizophrenia	Elevated in CSF and plasma of aging mice; impairs neurogenesis, cognition, memory; plasma levels elevated in AD and other forms of neurodegeneration	[38]
G-CSF	Granulocyte colony stimulating factor; CSF-3	Stimulates granulocyte activation, proliferation, survival, and differentiation, produced by endothelium and macrophages	Supports neuroprotection due to anti-apoptotic effects via pAKT activation	[39]
GM-CSF	Granulocyte-Macrophage Colony stimulating factor; CSF-2	Cytokine promotes host defenses; stimulates stem cells to produce granulocytes and monocytes-monocyte differentiate to macrophages and dendritic cells	Neuroprotective. Prevents neurodegeneration in MPTP models of PD; mediates autoimmune encephalitis	[40,41]

IFN-γ	Interferon-gamma; type II interferon	Pro-inflammatory cytokine and potent activator of macrophages; plays a role in mediating innate and adaptive immune responses; delayed immune response	Mediates delayed post-ischemia neurodegeneration via IFN-γ secreted by splenic macrophages; promotes inflammatory mediated impairment of neural stem and neuroprogenitor cell maturation and differentiation	[42-45]
IL-10	Interleukin-10; cytokine synthesis inhibitory factor	Anti-inflammatory cytokine; suppresses pro-inflammatory genes and cytokine secretion in macrophages and neutrophil	Neuroprotective; prevents LPS-induce neurodegeneration; expressed in microglia	[46]
IL-12 (p70)	Interleukin-12; p70 is the active heterodimer	Pro-inflammatory cytokine; antigen-induced expression in dendritic cells, macrophages, B cells and neutrophils; increases IFN-γ and TNF-α production; induces IL-7 in macrophages	Induces excitotoxic neuronal injury in brain by stimulating IL-7 in microglia	[47-50]
IL-13	Interleukin-13	Cytokine secreted by Th2 T helper cells; effects similar to those of IL-4 but with emphasis on reducing allergic inflammation; down-regulates Th2 helper functions; pro-inflammatory effects include increased IgE secretion by activated B cells	Potentially neuroprotective for cortical neurons; modulates cortical excitability; expression correlates with Aβ1-42 deposition in multiple sclerosis	[51,52]
IL-15	Interleukin-15	Pleiotropic pro-inflammatory cytokine, structurally similar to IL-2, produced by activated monocytes, macrophages, and dendritic cells. Promotes T cell proliferation and cytotoxicity via NK and cytotoxic T cells	Potential biomarker for AD due to elevated serum levels; produced by activated astrocytes	[23]
IL-17A	Interleukin-17A	Pro-inflammatory cytokine produced by T helper cells and induced by IL-23. Recruits monocytes and neutrophils to sites of inflammation; role in auto-immune diseases and microbial defenses	T-cell mediated delayed phase inflammatory injury in ischemic stroke	[53]

IL-1β	Interleukin-1beta; leukocyte pyrogen; leukocyte activating factor	Pro-inflammatory cytokine produced by activated macrophages; promotes p53-mediated apoptosis	Expressed by microglia in response to injury and exacerbates neuronal injury [IL-1]; causes excitotoxic neurodegeneration via increased glutamate, and MS progression via p53-mediated apoptosis; promotes oligodendrocyte cell death; also enhances synaptic transmission	[54-56]
IL-1ra	Interleukin-1 receptor antagonist; IL-1 inhibitor	Increases adhesion molecule expression; induces metalloproteinases and prostaglandins	Neuroprotective: Inhibits cytotoxic, ischemic, excitotoxic, and traumatic injury in the brain.	[56]
IL-2	Interleukin-2	Cytokine signaling regulator of activities in leukocytes responsible for immunity; increases T cell proliferation; activates B cells	Neuroprotective for maintaining septo-hippocampal cholinergic neurons; however, high levels cause cognitive dysfunction	[57]
IL-4	Interleukin-4	Cytokine induces differentiation of naïve T cells; regulates humoral and adaptive immune responses; anti-inflammatory actions reduce Th1, IFN-γ, macrophages, and dendritic cell IL-12	May regulate dopaminergic neuron functions; Effects are similar to those of IL-13.	[51]
IL-5	Interleukin-5	Pro-inflammatory cytokine; produced by Th2 T Helper cells; promotes activated B cell proliferation, maturation and immunoglobulin secretion	Induces proliferation and activation of microglia; increases nitrite production and probably nitrosative stress; serum levels elevated in major depressive disorders; utilizes neural plasticity-related RAS GTPase-extracellular signal-regulated kinase (Ras-ERK) pathway to mediate its effects on CNS plasticity	[58,59]
IL-6	Interleukin-6	Pro-inflammatory cytokine and anti-inflammatory myokine; induces B and T cell proliferation; induces protease inhibitor expression; secreted by macrophages and T cells to promote immune responses	Accumulates around senile plaques; levels elevated in AD PBMCs; Induced by MPTP in PD models; has paradoxical neuroprotective effects. Expressed in microglia	[60-62]

IL-7	Interleukin-7	Hematopoietic growth factor made by stromal, neuronal, dendritic, hepatocellular, and epithelial cells. Positive regulator of B and T cell development and differentiation	CNS and peripherally increased levels associated with autoimmune CNS diseases (MS/EAE) driven by increased levels of IL6, TNF-α, IFN-γ; enhances proliferation of myelin-activated T cells	[47]
IL-8	Interleukin-8	Chemokine ligand (C-X-C motif); regulates neutrophil migration by signaling through CXCR2; induces expression of proinflammatory proteases, MMP-2 and MMP-9; induces proapoptotic protein Bim (Bcl-2-interacting mediator of cell death) and cell death	Levels increased by brain injury; higher levels propagate secondary injury;	[54]
IL-9	Interleukin-9	Cytokine cellular signaling molecule that modulates pro-inflammatory responses, stimulating proliferation and inhibiting apoptosis; roles in autoimmune disease and asthma	Increased production in AD brain cells. Promotes T cell migration into the CNS	[62]
IP-10	Interferon gamma induced protein 10; CXCL10	Chemokine binds to cell surface CXCR3 receptors to activate chemoattraction of monocytes /macrophages, T cells, NK cells, and dendritic cells. Promotes T cell adhesion to endothelial cells, antitumor activity, and angiogenesis	Up-regulated in several neurodegenerative diseases and in MS; mediates stroke-induced neurodegeneration	[43]
MCP-1	Monocyte chemoattractant protein 1; CCL2 (chemokine motif ligand 2)	Chemokine anchored in the plasma membrane and secreted by monocytes, macrophages and dendritic cells, mainly in response to PDGF and CCR2 and CCR4 surface receptors; attracts monocytes	Induced in astrocytes by PDGF-BB; attracts monocytes promoting their transmigration through a disrupted blood-brain barrier Increased levels impair attention; executive function, and psychomotor speed.	[63]
MIP-1α	Macrophage inflammatory protein 1 alpha; Chemokine motif ligand 3 (CCL3)	Chemokine with chemoattraction for T cells, NK cells, monocytes, and immature dendritic cells; induces synthesis and release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α from fibroblasts and macrophages	Promotes neurodegeneration by attracting infiltration of microglia and macrophages; increased expression associated with spongiform neurodegeneration caused by oncornavirus	[64-66]

MIP-1β	Macrophage inflammatory protein 1 beta; Chemokine motif ligand 4 (CCL4)	Chemokine with chemoattraction for NK and T cells with actions similar to MIP-1α. Interacts with CCL3.	Impairs attention; executive function, psychomotor speed; increased expression with oncornavirus induced spongiform neurodegeneration	[64]
PDGF-BB	Plateled-derived Growth Factor-BB	Chemokine for monocytes and neutrophils; mitogenic for cells of mesenchymal origin; PDGF-B can homodimerize (PDGF-BB or heterodimerize with PDGF-A (PDGF-AB).	Neuroprotective; promotes neuronal survival; induces neurogenesis in dopaminergic neurons; however, also induces MCP-1 in astrocytes	[63,66,67]
RANTES	Regulated upon Activation, Normal T-cell Expressed, and Secreted	Chemokine with chemoattraction for T cells and leukocytes, promotes monocyte adhesion to endothelial cells. Binds to CCR1, CCR3, and CCR5 receptors	Major chemokine expressed in brain; including reactive astrocytes in mouse brains infected with scrapie virus; Potential neuroprotection after stroke, mediated by neuronal induction of neurotrophic factors in peri-infarct zones with attendant autocrine or paracrine supported neuronal survival	[68,69]
TNF-α	Tumor necrosis factor-alpha; cachexin	Pro-inflammatory cytokine of activated macrophages; binds to TNFR1; induces expression of other cytokines, chemokines (RANTES), metalloproteinases, adhesion molecules in acute phase responses; causes fever, cachexia, inflammation, and apoptosis. Anti-tumor and anti-viral effects. Dysregulated expression in cancer, psoriasis, and inflammatory bowel disease.	Dysregulated expression in neurodegeneration including AD, and in major depression. In neurodegeneration, TNF-α induces neuronal excitotoxic injury (via glutamate); accumulates around senile plaques; induced by MPTP; neuronal excitoxicity; also can increase synaptic transmission	[54,60,61]
VEGF	Vascular endothelial growth factor; vascular permeability factor (VPF)	Trophic factor in the PDGF subfamily; stimulates de novo vasculogenesis and angiogenesis, fibroblast proliferation, and monocyte/macrophage migration; restores oxygen supply to tissues injured by deprivation; increases microvascular permeability; levels elevated in diabetes and cancer.	CSF levels elevated in normal brain aging; may be neuroprotective, reduced CNS/CSF levels correlate with hippocampal atrophy, loss of executive functions and memory; interactive effect with Aβ1-42	[70]

Statistics

Initial analyses compared results in the control, MCI and AD groups (Supplementary Tables 1 and 2). However, since the AD biomarkers and both serum and CSF inflammatory profiles (responses) were similar in the MCI and AD groups, the data presentation was simplified by pooling data from the AD and MCI groups for comparison with controls. Statistical analyses were performed using NCSS version 11 (Kaysville, UTAH) and Stata 14 (Stata Corp, College Station, Texas). Statistical comparisons among AD, MCI and control groups were performed using one-way repeated measures analysis of variance (ANOVA) and the post hoc Tukey-Kramer Multiple Comparison Test of significance. Comparisons between the combined AD/MCI and control groups were made using unpaired two-tailed Student's *t*-tests with 4% false discovery corrections. The level of statistical significance was defined as $P< 0.05$.

Results

Study Groups: Paired serum and CSF samples were available from 21 control subjects and 18 subjects with MCI or AD. Demographic characteristics are provided in Table 2. The mean age (± S.D.) of the control group (45.6 ± 11.8) was significantly lower than the MCI (69.1 ± 7.2) and AD (67.5 ± 11.3) groups (P<0.0001), whereas there was no significant age difference between the MCI and AD groups. The control group had 11 males and 10 females. The MCI group had 7 males and 1 female. The AD group had 5 males and 5 females. At the time samples were collected, the mean (± S.D.) Mini-Mental State Examination (MMSE) scores were 26.4 ± 3.1 (range 21-30) and 21.9 ± 5.5 (range 13-28) in the MCI and AD groups respectively. The mean MMSE score was significantly lower in the AD relative to the MCI group (P<0.05). MMSE scores were not obtained for control subjects.

Table 2: Subject Demographics

	Control	MCI	AD
Number Subjects	21	8	10
Age: Years ± S.D (Range)	45.6 ± 11.8 (28-77)	69.1 ± 7.2 (59-77)	67.5 + 11.3 (49-83)
Sex: M/F	11/10	7/1	5/5
MMSE Score ± SD (Range)	N.D.	26.4 ± 3.1 (21-30)	21.9 ± 5.5 (13-28)
Comparisons of mean ages, sex ratios, and MMSE scores among subjects diagnosed as control, MCI, or AD. MMSE scores were not obtained (N.D.) for control subjects.			

Biomarker Assay Results

$A\beta_{1-42}$ and pTau were measured in serum and CSF by direct binding ELISAs and 3-way inter-group comparisons were made by repeated measures ANOVA and the post hoc Tukey multiple comparisons test (Figure 1). Significant inter-group differences were detected for the mean levels of serum pTau, serum and CSF $A\beta_{1-42}$ and the CSF/Serum ratios of pTau and $A\beta_{1-42}$, whereas a trend effect was detected for CSF pTau (Figure 1A). Serum pTau levels were similarly high in control and MCI samples whereas in AD the mean levels were significantly reduced relative to MCI (P=0.04) and control (P=0.002) (Figure 1B). The mean levels of pTau in CSF gradually but not significantly increased from control to MCI and then AD. The mean serum levels of $A\beta_{1-42}$ were significantly reduced in both the MCI and AD groups relative to control (P<0.0001), although the mean MCI level was somewhat (but not significantly) higher than in the AD group (Figure 1C). In contrast, the mean CSF $A\beta_{1-42}$ levels were similarly and significantly elevated in the MCI and AD groups relative to control (P=0.01) (Figure 1C). The calculated mean CSF/serum ratios of pTau and $A\beta_{1-42}$ progressively increased from control to MCI and then AD, resulting in

significantly higher CSF:serum ratios of pTau (P=0.0002) and $A\beta_{1-42}$ (P=0.006) in AD relative to control, but no significant differences between AD and MCI (Figure 1D). These findings correspond with progressively reduced $A\beta_{1-42}$ and pTau clearance from the brain with advancing neurodegeneration from control to MCI and MCI to AD.

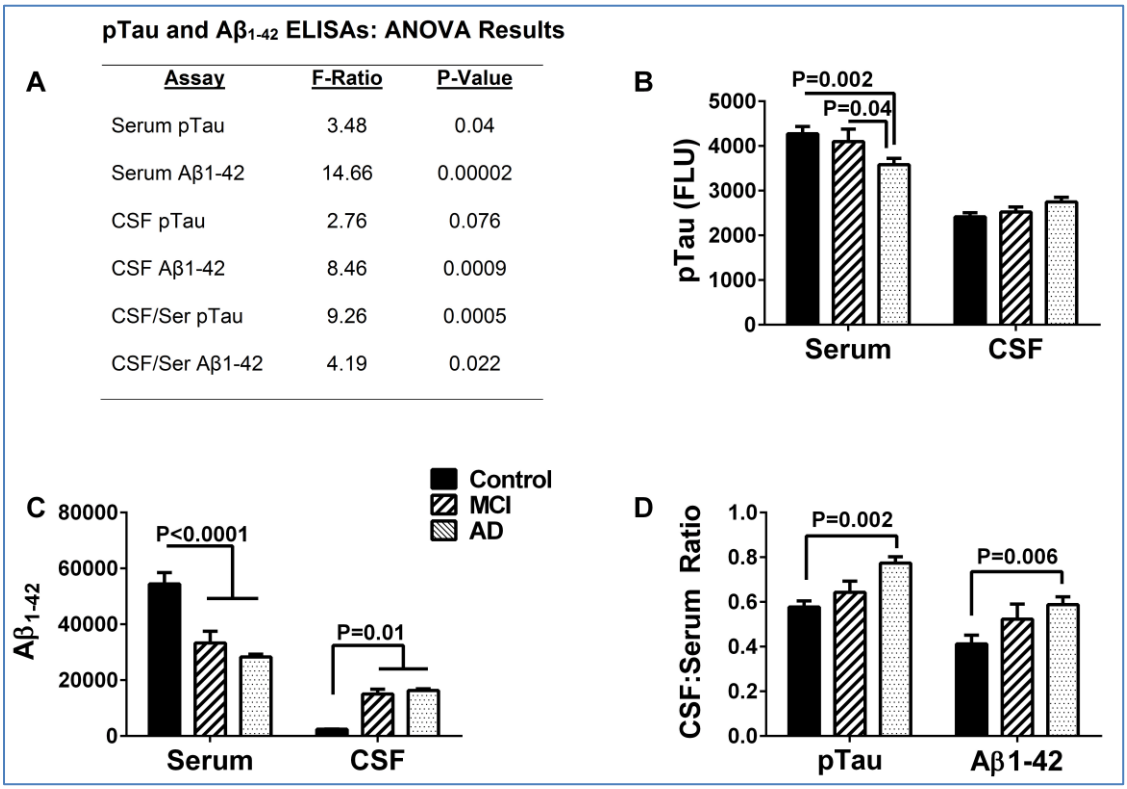

Figure 1: AD Biomarkers

Figure 1: AD Biomarkers: Amyloid-beta peptide ($A\beta_{1-42}$) and phospho-tau (pTau-307) immunoreactivities were measured in paired serum and CSF samples from control, MCI, and AD subjects using direct binding ELISAs. Immunoreactivity was detected with horseradish peroxidase-conjugated secondary antibody and the Amplex UltraRed fluorophore. Fluorescence intensity was measured (Ex 565 nm/Em 595 nm) in a SpectraMax M5 microplate reader. (A) Data were analyzed by repeated measures ANOVA. Graphs depict mean ± S.D. of (B) pTau, (C) $A\beta_{1-42}$, and (D) CSF:Serum ratios of pTau and $A\beta_{1-42}$ with significant differences obtained by the post hoc Tukey-Kramer multiple comparisons test.

Peripheral Indices of Inflammation

Initial analyses compared control with MCI and AD by one-way repeated measures ANOVA and post hoc Tukey-Kramer multiple comparisons tests (Supplementary Table 1). Among the 27 factors measured in serum by multiplex ELISA, 12 (44.4%) showed significant inter-group differences and 4 (14.8%) had statistical trends. The significant or trend effects of AD and MCI were directionally concordant for 15 of the 16 factors (93.8%); the one exception was b-FGF which was significantly reduced in AD and unchanged in MCI relative to control. Therefore, the data analysis and presentation were simplified by combining results from the MCI and AD groups for comparison with controls.

Two-tailed *t*-tests with 4% false discovery corrections detected significant inter-group differences for 15 of the 27 factors (55.6%) including b-FGF, GM-CSF, IL-15, IL-1β, IL-2, and MCP-1 which were reduced in the MCI/AD group, and eotaxin, IL-13, IL-17A, IL-4, IL-5, MIP-1α, PDGF-BB, RANTES, and TNF-α, which were increased in

MCI/AD relative to control (Table 3). In contrast, no significant inter-group differences were observed with respect to G-CSF, IFN-γ, IL-10, IL-12p70, IL-1ra, IL-6, IL-7, IL-8, IL-9, IP-10, MIP-1β, and VEGF. The higher levels of IL-1β and TNF-α and unchanged levels of IFN-γ, IL-8, and IL-10 in MCI/AD are consistent with previous findings, whereas the increased levels of IL-4, increased levels of IL-2 and unchanged levels of IL-6 are discordant with the findings in a meta-analysis [71].

Table 3: Serum Cytokine Levels

Factor	Control (Mean ± S.D.)	MCI/AD (Mean ± S.D.)	P-Values
b-FGF	28.18 ± 3.40	23.99 ± 3.59	0.01
Eotaxin	53.41 ± 14.84	79.55 ± 27.42	0.0005
G-CSF	33.37 ± 27.19	25.44 ± 3.42	
GM-CSF	25.98 ± 10.13	12.97 ± 3.36	<0.0001
IFN-γ	67.07 ± 31.32	65.40 ± 7.55	
IL-10	6.18 ± 5.337	5.13 ± 2.80	
IL-12p70	10.76 ± 15.94	9.60 ± 2.78	
IL-13	0.90 ± 0.6595	1.81 ± 1.13	<0.0001
IL-15	11.37 ± 5.092	3.64 ± 2.44	<0.0001
IL-17A	19.96 ± 5.508	28.88 ± 4.88	<0.0001
IL-1β	1.87 ± 0.3285	2.10 ± 0.27	0.0080
IL-1ra	89.15 ± 43.88	82.24 ± 31.83	
IL-2	3.77 ± 1.209	2.04 ± 0.94	<0.0001
IL-4	1.30 ± 0.235	1.93 ± 0.21	<0.0001
IL-5	5.56 ± 1.78	14.78 ± 2.46	<0.0001
IL-6	12.35 ± 22.17	10.78 ± 0.64	
IL-7	2.41 ± 2.956	2.05 ± 0.68	
IL-8	5.68 ± 4.162	4.39 ± 0.81	
IL-9	7.78 ± 3.086	12.14 ± 14.15	
IP-10	1582.00 ± 2555	363.30 ± 254.00	
MCP-1	44.77 ± 52.07	18.07 ± 3.56	0.0001
MIP-1α	1.37 ± 0.2346	1.56 ± 0.14	0.0010
MIP-1β	29.34 ± 20.03	31.23 ± 13.79	
PDGF-BB	321.10 ± 265.1	853.90 ± 243.90	<0.0001
RANTES	1855.00 ± 233.3	2130.00 ± 183.00	<0.0001
TNF-α	31.48 ± 5.514	38.01± 5.05	0.0006
VEGF	12.51 ± 5.527	13.17± 5.68	

Serum samples from 21 control and 18 MCI/AD subjects were assayed for 27 cytokines and chemokines using a multiplex bead-based ELISA (See Table 1 for full names and functions). Fluorescence intensity was measured in a MAGPIX, and cytokine/chemokine concentrations (pg/mL) were software-generated (BioRad) from standard curves. Inter-group comparisons were made using unpaired, two-tailed *t*-tests with 4% false discovery rate corrections. Significant P-values (P<0.05) and statistical trends (0.05<P<0.10; italicized) are shown. See Supplementary Table 1 for the 3-way statistical comparisons among the AD, MCI and control groups.

To simplify the data presentation and analysis, fold-differences in the levels of cytokines, chemokines, and trophic factors in the MCI/AD group relative to controls were calculated and displayed using a databar plot (Figure 2). Bars to the left of the vertical axis reflect MCI/AD-associated reductions in factor expression, whereas bars to the right indicate increases in factor levels relative to control. Differences of 5% or less were generally regarded as neutral or unchanged relative to control. Significant P-values are displayed to the right of the databars.

The databar plot revealed that MCI/AD was associated with reduced serum levels of IP-10, IL-15, MCP-1, GM-CSF, IL-2, G-CSF, IL-8, IL-10, IL-7, IL-6, IL-12p70, and b-FGF although the differences from control were significant for only IL-15, MCP-1, GM-CSF, IL-2 and b-FGF (Figure 2). Despite the large fold-change for IP-10, the statistical comparison was not significant due the large standard deviations. In contrast, the relatively small inter-group differences in serum b-FGF levels were significant because the variances were tight. The MCI/AD had significantly increased mean serum levels of PDGF-BB, IL-5, IL-13, eotaxin, IL-4, IL-17A, TNF-α, RANTES, and MIP-1a, and IL-1β, non-significant increases in IL-9, and no significant alterations in MIP-1β, VEGF, IFN-γ, or IL-1ra (Figure 2). Therefore, 10 (37.3%) serum cytokine, chemokine, and trophic factor levels were significantly increased and 5 (18.5%) were significantly decreased in MCI/AD relative to control.

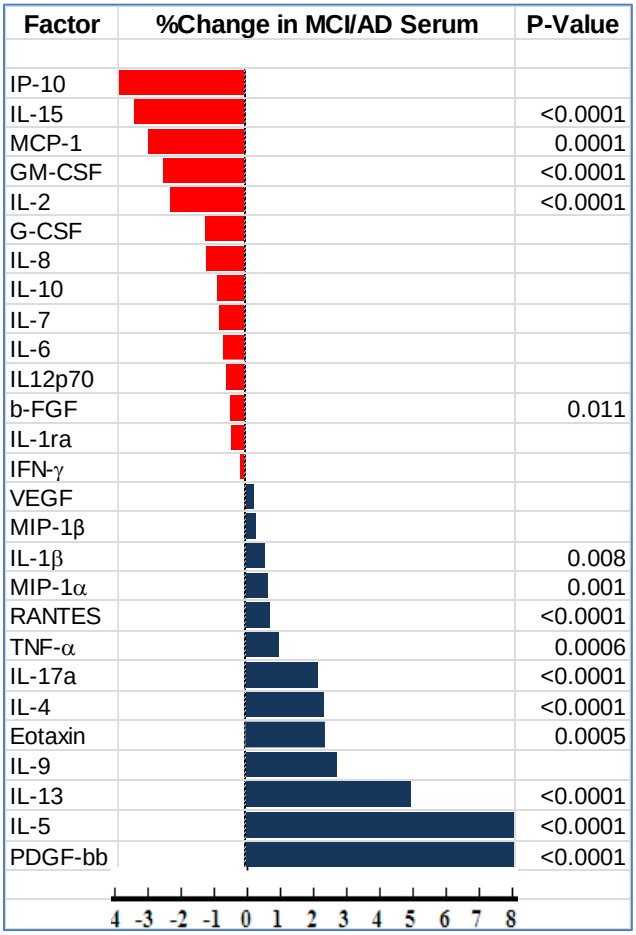

Figure 2: Databar Display of MCI/AD Effects on Serum Cytokine Profiles

Figure 2: Databar Display of MCI/AD Effects on Serum Cytokine Profiles**:** Bead-based multiplex ELISAs were used to measure levels of 27 pro-inflammatory cytokines and chemokines (Left column; see Table 1 for full names and functions) in serum. Captured antigens were detected with phycoerythrin-labeled secondary antibodies and the plates

were read in a MAGPIX. Direct comparisons of the levels of immunoreactivity are presented in Table 3 (2-way: control, MCI/AD) and Supplementary Table 1 (3-way: control, MCI, AD). The calculated mean percentage differences in levels of immunoreactivity between MCI/AD and control results are displayed such that reductions are represented by bars to the left and increases by bars to the right. The digits on the bottom ruler correspond to 20% incremental reductions (minus numbers to the left of 0) or increases (plus numbers to the right of 0) in cytokine expression in MCI/AD relative to control. Significant differences are shown in the right column.

CSF Cytokines/Chemokines

Initial comparisons among the control, MCI and AD groups using one-way repeated measures ANOVA and post hoc Tukey-Kramer multiple comparisons tests revealed just 8 factors that were significantly altered in MCI and/or AD relative to control, including b-FGF, GM-CSF, IL-13, IL-17A, IL-5, IL-7, PDGF-BB, and VEGF (Supplementary Table 2). Although the significant directional shifts were largely (6 of 8; 75%) the same for MCI and AD, the two discordances were due to significantly increased levels of IL-13 in AD and not MCI, and increased IL-7 in MCI but not AD. As was done for the serum assays, results from the MCI and AD groups were combined to simplify the data analysis and presentation (Figure 3).

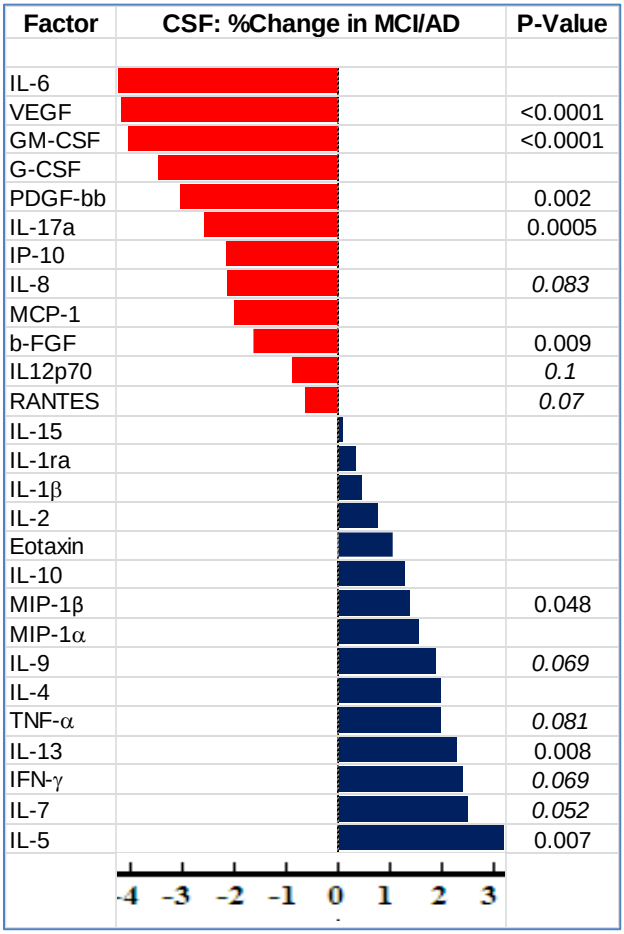

Figure 3: Databar Display of MCI/AD Effects on CSF Cytokine Profiles

Figure 3: Databar Display of MCI/AD Effects on CSF Cytokine Profiles. Bead-based multiplex ELISAs were used to measure levels of 27 pro-inflammatory cytokines and chemokines (Left column; see Table 1 for full names and functions) in CSF. Captured antigens were detected with phycoerythrin-labeled secondary antibodies and the plates

were read in a MAGPIX. Direct comparisons of the levels of immunoreactivity are presented in Table 4 (2-way: control, MCI/AD) and Supplementary Table 2 (3-way: control, MCI, AD). The calculated mean percentage differences in levels of immunoreactivity between MCI/AD and control results are displayed such that reductions are represented by bars to the left and increases by bars to the right. The digits on the bottom ruler correspond to 20% incremental reductions (minus numbers to the left of 0) or increases (plus numbers to the right of 0) in cytokine expression in MCI/AD relative to control. Significant differences and statistical trends (0.05<P<0.10) are listed in the right column.

Table 4: CSF Cytokine Levels

Factor	**Control (Mean ± S.D.)**	**MCI/AD (Mean ± S.D.)**	**P-Values**
b-FGF	17.43 ± 4.29	12.14 ± 5.88	0.0090
Eotaxin	14.00 ± 5.94	16.74 ± 9.59	
G-CSF	65.13 ± 194.40	22.75 ± 10.01	
GM-CSF	71.19 ± 25.07	17.23 ± 15.32	<0.0001
IFN-γ	48.45 ± 23.14	70.34 ± 39.35	*0.0690*
IL-10	4.48 ± 2.04	5.56 ± 2.47	
IL-12p70	2.90 ± 1.74	2.43 ± 2.82	*0.1000*
IL-13	23.81 ± 9.42	34.03 ± 12.31	0.0080
IL-15	16.28 ± 6.40	16.56 ± 3.04	
IL-17A	8.07 ± 3.32	4.17 ± 3.91	0.0005
IL-1β	0.89 ± 0.91	0.96 ± 0.69	
IL-1ra	16.01 ± 10.53	17.00 ± 9.84	
IL-2	2.01 ± 0.70	2.29 ± 1.25	
IL-4	1.97 ± 1.02	2.70 ± 1.79	
IL-5	3.52 ± 2.10	5.65 ± 2.52	0.0070
IL-6	59.54 ± 142.50	11.98 ± 5.75	
IL-7	3.30 ± 1.85	4.83 ± 2.63	*0.0520*
IL-8	43.42 ± 93.44	26.03 ± 6.96	*0.0830*
IL-9	4.82 ± 1.98	6.52 ± 2.90	*0.0690*
IP-10	2441.00 ± 5308.00	1450.00 ± 3198.00	
MCP-1	237.00 ± 275.00	148.40 ± 33.91	
MIP-1α	0.98 ± 0.45	1.26 ± 0.71	
MIP-1β	7.59 ± 5.37	9.57 ± 4.04	0.0480
PDGF-BB	3.52 ± 1.99	1.51 ± 1.67	0.0020
RANTES	7.38 ± 4.41	6.51 ± 8.02	*0.0700*
TNF-α	6.80 ± 3.96	9.33 ± 4.92	*0.0810*
VEGF	11.87 ± 4.60	2.55 ± 2.92	<0.0001

CSF samples from 21 control and 18 MCI/AD subjects were assayed for 27 cytokines and chemokines using a multiplex bead-based ELISA (See Table 1 for full names and functions). Fluorescence intensity was measured in a MAGPIX, and cytokine/chemokine concentrations (pg/mL) were software-generated (BioRad) from standard curves. Inter-group comparisons were made using unpaired, two-tailed *t*-tests with 4% false discovery rate corrections. Significant P-values (P<0.05) and statistical trends (0.05<P<0.10; italicized) are shown. See Supplementary Table 2 for the 3-way statistical comparisons among the AD, MCI and control groups.

In contrast to serum in which the levels of 55.6% of the factors were significantly altered in MCI/AD, two-tailed *t*-tests with 4% false discovery corrections detected significant inter-group differences for just 8 of the 27 CSF factors (29.6%) including b-FGF, GM-CSF, IL-17A, PDGF-BB and VEGF, which were reduced, and IL-13, IL-5, MIP-1β, PDGF-BB, and VEGF, which were increased in MCI/AD relative to control (Table 4). In addition, MCI/AD-associated statistical trend ($0.05 \leq P \leq 0.10$) reductions in CSF cytokine/chemokine levels were observed for IL-12p70, IL-8, and RANTES, whereas trend increases occurred for IFN-γ, IL-7, IL-9 and TNF-α. No significant MCI/AD versus control inter-group differences were observed with respect to the CSF levels of eotaxin, G-CSF, IL-10, IL-15, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IP-10, MCP-1, or MIP-1β (Table 4 and Figure 3).

To facilitate comparisons with the serum responses and the data interpretation, fold-differences in the CSF levels of cytokines, chemokines, and trophic factors in the MCI/AD group relative to controls were calculated and displayed using a databar plot (Figure 3) as described above. The databar plot revealed that MCI/AD was associated with reduced CSF levels of IL-6, VEGF, GM-CSF, G-CSF, PDGF-BB, IL-17A, IP-10, IL-8, MCP-1, b-FGF, IL-12p70 and RANTES. MCI/AD had significant fold increases in CSF IL-5, IL-13, and MIP-1b, and trend increases in IL-7, IFN-γ, TNF-α, and IL-9 (Figure 3). Although the percentage reductions in CSF levels of IL-6, G-CSF, IP-10, MCP-1 and increases in IL-4, MIP-1a, IL-10, eotaxin, and IL-2 overlapped with other responses that reached statistical significance or a statistical trend, the presence of outlier points reflects the non-uniform responses in both the MCI/AD and control groups.

Concordant Serum and CSF Inflammatory Responses in MCI/AD

To gauge whether the inflammatory responses in CSF and serum were related or independent, we compared the rates of concordant and discordant increases or reductions in cytokine/chemokine levels in the MCI/AD versus control group. If the CSF and serum levels were both increased or reduced by at least 10%, or both were unchanged (less than 10%) relative to control, the responses were scored as concordant. Otherwise, the responses were scored as discordant. MCI/AD CSF and serum samples showed concordant reductions in GM-CSF, G-CSF, IP-10, IL-8, MCP-1, and IL-12p70, increases in IL-5, IL-13, TNF-α, IL-4, IL-9, MIP-1α, and eotaxin, and neutral responses for IL-1β and IL-1ra. Therefore, among the 27 factors, 16 (59.2%) showed similar responses and trends in CSF and serum of the MCI/AD subjects. Importantly 5 (IL-5, TNF-α, IL-9, MIP-1α, and eotaxin) of the 7 cytokines/chemokines that were concordantly elevated in CSF and serum have clear pro-inflammatory effects both systemically and in the CNS (see Table 1). Anti-inflammatory cytokines IL-4 and IL-13, were significantly elevated in serum as well as CSF. At the same time, 6 pro-inflammatory cytokines and chemokines (GM-CSF, G-CSF, IP-10, IL-8, IL-12p70, and MCP-1) or chemokines were concordantly reduced in CSF and serum, although the dual responses were only significant for GM-CSF. Therefore, the main responses in MCI/AD were to significantly increase or decrease systemic pro-inflammatory factors, vis-à-vis similar but less pronounced responses in CSF. The exceptions were IL-5 and IL-13 which were similarly increased, and GM-CSF which was similarly decreased in serum and CSF of MCI/AD subjects relative to control. Among the trophic/angiogenesis factors, b-FGF was significantly reduced in both CSF and serum (Tables 3 and 4 and Figures 2 and 3).

Discordant Serum and CSF Inflammatory Responses in MCI/AD

Discordant MCI/AD CSF and serum responses were observed for the cytokines, IL-2, IL-6, IL-10, IL-15, IL-17A, and IFN-γ, the chemokines MIP-1β, PDGF-BB, and RANTES, and trophic factors IL-7 and VEGF (Figures 2 and 3). Among the cytokines, two pro-inflammatory (IL-2 and IL-15) were significantly reduced in serum but increased or unchanged in CSF, whereas IL-17A was increased in serum but decreased in CSF. Among the chemokines, IFN-γ and MIP-1β were unchanged in serum but significantly elevated in CSF, while PDGF-BB and RANTES were significantly

increased in serum and reduced in CSF (Figures 2 and 3). In regards to the trophic factors, VEGF was sharply reduced in CSF but unchanged in serum, and IL-7 was modestly reduced in serum but had a trend increase in CSF. Therefore, in MCI/AD, the neuro-inflammatory profiles were dissimilar to those in serum for 11 of the 27 (40.7%) cytokines, chemokines and trophic factors examined at the same time point.

To further address the potential relation between peripheral and central changes in inflammation, within-group Pearson correlation analyses were performed between serum and CSF levels of the inflammatory indices. When corrected for multiple comparisons using the Bonferroni method, there were no significant correlations among the MCI/AD patients, and only 2 inflammatory markers (GM-CSF r=0.9763, P=0.01 and MCP-1, r=0.8643; P=0.01) were significant in the control group. These results suggest that the inflammatory processes in the CSF and serum were concurrent but non-identical and possibly independent.

Discussion

This study uniquely examined paired, fresh frozen serum and CSF samples and demonstrated that systemic and CNS inflammatory indices are simultaneously altered in MCI and AD. Corresponding with previous reports, the MCI and AD cases included in our study had elevated CSF/serum ratios of pTau and $A\beta_{1-42}$, indicating reduced clearances from the brain [72,73]. Furthermore, the graded responses from MCI to AD is consistent with the concept that CNS clearance of pTau and $A\beta_{1-42}$ declines with disease progression. One of the main goals of this research was to determine if both systemic and neuro-inflammation were present in MCI and AD, and if so, were their profiles identical or distinct and independent. Since the CSF and serum pTau and $A\beta_{1-42}$ levels and responses of the 27 cytokines and chemokines were similarly altered in MCI and AD relative to control, the MCI and AD results were pooled to simplify the data presentation and comparisons with control.

The MCI/AD subjects had broadly activated pro-inflammatory pathways as evidenced by the elevated mean serum levels of multiple pro-inflammatory cytokines and chemokines. The elevated levels of MIP-1α, RANTES, IL-4, IL-5, IL-1β, TNF-α, IL-13, and eotaxin would serve to activate and propagate systemic inflammatory responses and tissue injury cascades. The MCI/AD-associated serum elevations of IL-1β, IL-4, and TNF-α are consistent with a meta-analysis study demonstrating pro-inflammatory cytokine activation in peripheral blood of people with AD [71], and also data summarized in a recent review article [60]. However, one discrepancy is that in contrast to those previous reports, we did not detect significantly increased levels of IL-6 in the MCI/AD sera.

The elevated peripheral blood levels of IL-1β and TNF-α are of particular interest because both cytokines have been linked to neurodegeneration, including AD [60]. High levels of IL-1 may be important for initiating injury, degeneration, and cell death, self-reinforcing cascades that lead to progressive death of neurons [64]. Mechanistically, IL-1 activation of astrocytes with attendant increased S100b expression leads to neuritic dystrophy with synaptic disconnection, increased neuronal production of $A\beta_{1-42}$, intracellular calcium, and excitotoxic cell death [54-56,74]. Increased production and accumulation of $A\beta_{1-42}$ activates microglia and further increases IL-1β and IL-6 [20,75]. Similarly, TNF-α induces neuronal injury [54,60,61], functioning as a key regulator of pro-inflammatory cascades that impair neuronal viability, synaptic integrity, and gene expression, and its levels are elevated in many neurodegenerative disease including AD, Parkinson's disease, and motor neuron disease [76]. Increased levels of IL-1β and TNF-α correspond with cognitive impairment and the presence of typical histopathological lesions of AD [76]. Although previous studies have shown that both IL-1β and TNF-α are elevated in AD brains, neuro-inflammatory responses can be generated from systemic sources since pro-inflammatory cytokines, including TNF-α and IL-1β, can cross the blood-brain barrier via active transport mechanisms [77-81]. Therefore, the significantly elevated levels of IL-1β and TNF-α in serum and modest or neutral responses in CSF of the MCI/AD subjects support the concept that

systemically-derived inflammatory responses can mediate CNS neuro-inflammation in the early stages of neurodegeneration.

Apart from the known alterations in peripheral blood cytokines and chemokines, this study demonstrates significantly elevated levels of MIP-1, RANTES, IL-17A, IL-5, IL-13, eotaxin and PDGF-BB, whose functions are to attract inflammatory cells, including those involved in T cell, B cell, dendritic cell, monocyte/macrophage, and allergic responses, or in the cases of MIP-1a and IL-13, reinforce the actions of cytokines that were previously shown to be increased in AD peripheral blood (Table 1). On the other hand, this study detected reduced MCI/AD serum levels of the pro-inflammatory chemokines, IP-10 and MCP-1, and the pro-inflammatory cytokine, IL-15. One function of IP-10, like VEGF and b-FGF, is angiogenesis. Since b-FGF was also significantly reduced and VEGF levels were un-altered, one potential interpretation of the IP-10/b-FGF findings is that peripheral factors mediating angiogenesis are suppressed in MCI and AD. Understanding the contributions of these alterations to micro-vascular dysfunction in AD requires further study. On the surface, the reduction in MCP-1 seems contradictory. However, it is noteworthy that all the pro-inflammatory cytokines and chemokines which were elevated in MCI/AD sera function by activating T cells via Th2 helper cells, whereas MCP-1 activates Th1 cytotoxic T cells (Table 1). Conceivably, systemic inflammation in MCI/AD is mediated by activated T helper cells, via IL-5, IL-13, and IL-17A, and not cytotoxic T cell responses, accounting for the reduced serum levels of MCP-1.

In CSF, the significantly increased levels of IL-5 and IL-13 were concordant with results obtain for serum, confirming a role for Th2 helper cell-mediated neuro-inflammation in AD. The increased CSF level of IL-7 is of interest because of IL-7 promotes proliferation of myelin-activated T cells [47]. Since white matter degeneration is an early finding in AD [82,83], the discordantly elevated IL-7 in CSF but not serum suggests that this aspect of neuro-inflammation may be distinct and selective. Alternatively, since IL-7 is driven by increased levels of IL-6, TNF-α, and IFN-γ [47], elevated serum (systemic) levels of TNF-α may help drive IL-7-linked neuro-inflammation and white matter degeneration. If correct, this concept would support the use of anti-inflammatory agents and TNF-α inhibitors to block IL-7-mediated myelin degeneration in the early stages of AD.

PDGF-BB is neuroprotective, promoting neuronal survival and neurogenesis [63,66,67]. Reduced levels of PDGF-BB in MCI/AD CSF suggest that anti-survival and anti-growth pathways are activated early in neurodegeneration. Similarly, the reduced levels of b-FGF and VEGF point to impairments in neuroprotection and growth signaling [29,37,70], which could inhibit angiogenesis needed to support micro-vascular perfusion. Altered b-FGF expression could differentially impact the brain at different stages of neurodegeneration since in addition to its neurotrophic/ neuroprotective actions, b-FGF levels increase in the later stages of AD and in association with senile plaques, neurofibrillary tangles and neuropil threads [37,84]. VEGF also has neuroprotective effects in normal aging, but with neurodegeneration, VEGF levels in CSF and brain decline [70]. Reduced VEGF expression correlates with hippocampal atrophy, loss of executive function, and decline in memory [70]. It is not known whether these phenomena represent causes or reactions to neurodegeneration. However, the early disease stage reductions in CSF PDGF-BB, b-FGF, and VEGF observed herein suggest that these responses reflect impairments in neuronal survival and growth. Whether selective CNS modulation of PDGF-BB, b-FGF, and VEGF is mediated by endogenous or systemically derived inflammatory and immune factors is not known.

The concept that endogenous neuro-inflammation can also drive neuronal loss and dysfunction in neurodegeneration is supported by the finding that IL-17 was reduced in MCI/AD CSF but not serum. In the brain, astrocyte production of IL-17 is neuroprotective and inhibits apoptosis [85]; therefore, the reduced CSF levels of IL-17 in MCI/AD were likely injurious or permissive to neuronal cell death. In contrast, systemic IL-17 has pro-inflammatory actions and reduced levels are protective [85,86]. Although the sources of altered cytokine and

chemokine expression in CSF were not identified in this study, there is ample evidence that cytokines and chemokines can be derived from microglia and astrocytes. The activated microglia and astrocytes could serve to attract migration of T cells to the CNS, and under those circumstances, release cytokines and chemokines into the CSF, including with neurodegeneration. Evidence supporting the concept that activities in the CNS such as neuronal or endothelial activation via environmental cues can generate regional gateways through which pathogenic T cells are attracted and cause CNS injury was recently reviewed [87].

The final consideration is the degree to which the systemic (serum) and neuro-inflammatory (CSF) responses were concordant or discordant. Concordant responses in which pro-inflammatory cytokine and chemokine levels in MCI/AD were strikingly more elevated in serum than CSF would support the concept that systemic inflammation drives neuro-inflammation and neurodegeneration. Among the 27 factors examined, more than half (55.6%) were significantly modulated in MCI/AD serum compared with 29.6% in CSF. Although the findings that 9 factors were concordantly increased and 7 were concordantly reduced in serum and CSF could indicate that some underlying factors driving systemic and CNS inflammation are shared in MCI/AD, a potential causal relationship between peripheral and CNS inflammatory responses cannot be excluded. Evidence supporting the concept that peripheral or systemic inflammatory responses drive CNS inflammatory injury has been provided in experiments in which CNS autoimmune inflammation was prevented by impairment of T cell trafficking across the blood-brain barrier [88], or inhibition of IL-17A-induced disruption of the BBB [89]. Of note is that in regard to insulin resistance diseases, ample data support the concept that systemic pathology mediated by obesity, diabetes mellitus, non-alcoholic fatty liver disease, or metabolic syndrome promotes or exacerbates CNS disease and leads to cognitive impairment [90-95].

Discordant responses observed with respect to 11 cytokines/chemokines that were elevated in serum but minimally increased or reduced CSF, or reduced in serum but elevated in CSF do not support a causal role for systemic inflammation in the pathogenesis of neuro-inflammation. Instead, the findings suggest that either the systemic and CNS processes leading to inflammation are not identical, or that systemic and CNS responses to the same pathogenic processes are differentially regulated. Moreover, discordant neuro-inflammatory responses vis-à-vis absent or inhibited systemic inflammation would argue in favor of brain-specific processes causing cognitive impairment. Correspondingly, the failure to detect any significant within-subject correlations between serum and CSF cytokines/chemokines in the MCI/AD group suggests that at the disease stages examined, the systemic and neuro-inflammatory responses were unrelated. However, it does not exclude potential links at earlier stages of cognitive decline since in the control group, serum and CSF levels of GM-CSF and MCP-1 were significantly correlated.

One of the main strengths of this study was the ability to simultaneously assay paired serum and CSF samples to assess alterations in systemic and neuro-inflammatory profiles in MCI/AD relative to control subjects, and differential patterns of cytokine/chemokine activation in peripheral blood and brain at relatively early stages of neurodegeneration. The results provide evidence that neuro-inflammation is accompanied by systemic inflammatory responses in MCI/AD. However, the data also hint at dual mechanisms of neuro-inflammation in MCI/AD in which some aspects may be driven by systemic factors (inflammation) whereas others are likely to be endogenous and specific to the brain. The latter phenomenon could account for the failure of anti-inflammatory mediators to modify the course of disease in AD [96,97] since many of those treatments may have had limited ability to cross the blood-brain barrier [98].

The cross-sectional nature of the study with only single time-point samples and small group sizes limit interpretation of the results. The significant difference in mean age may also have contributed to some of the inter-group differences. Furthermore, the study design did not permit us to determine the sources of cytokine/chemokine

activation in peripheral blood, e.g. peripheral blood leukocytes, liver, or other tissues. Since this study did not include analysis of cytokine polymorphisms, no conclusions can be drawn regarding potential genetic factors driving these pro-inflammatory responses. Future studies should include longitudinal paired assessments of the status and nature of systemic and CNS inflammatory responses in aging, MCI, transition phases to AD, and with AD progression. In addition, efforts should be made to determine if the same or unrelated factors mediate systemic and CNS inflammation in MCI and AD.

Conflict of Interest

All authors declare no actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. Our institutions do not have contracts relating this research through which it or any other organization may stand to gain financially now or in the future. There are no other agreements of authors or their institutions that could be seen as involving a financial interest in this work.

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