Published in final edited form as:

Exp Gerontol. 2010 January ; 45(1): 57–63. doi:10.1016/j.exger.2009.08.003.

Biomarkers of inflammation and amyloid- β phagocytosis in patients at risk of Alzheimer disease

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Abstract

The ultimate goal of diagnostic research is a blood test detecting the risk of Alzheimer disease (AD) before neuronal damage develops. Current amyloid- β (A β) tests do not detect the process leading to neurodegeneration. Novel immunologic and proteomics tests are based on aberrant appearance of inflammatory cytokines in the CSF and other protein biomarkers in the CSF or blood, and immune biomarkers of peripheral blood mononuclear cells (PBMC's). Cytokines, chemokines, complement factors, serum amyloid P component, and signaling proteins in the CSF or blood may be a rich source of diagnostic biomarkers, but the power of these tests will need to be examined in prospective studies. Recently-described flow cytometric test of defective A β phagocytosis detects patients with AD with a high sensitivity and specificity in distinct populations (confirmed AD patients vs. active University professors), but further experience is necessary for its use in general population at risk of AD. The analysis of the transcriptome of peripheral blood mononuclear cells "stressed" by A β is beginning to unravel the relations between specific pathways and AD. Thus novel diagnostic tests may provide biomarkers for pre-clinical detection, clarification of progression from MCI to AD, and follow-up of patients in clinical trials of immunostimulating therapies.

Keywords

Alzheimer disease biomarkers; amyloid-beta; macrophage; microglia; astrocyte; phagocytosis; inflammation; serum amyloid P; complement; neuron

1. Introduction

The quest for effective prevention of AD requires detection of the risk of future dementia with high sensitivity and specificity in the pre-clinical phase before neuronal damage develops. This period may be several decades, as suggested by the Nunn study (Riley, et al., 2005). The search for biological biomarkers has established cerebrospinal fluid (CSF) biomarkers, low A β 1-42 and high phosphorylated- τ 181 (P- τ) as markers of the state, but not the process, of neurodegeneration (Sunderland, et al., 2003) (Blennow and Hampel, 2003). Low A β and high

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τ or P- τ in CSF have been confirmed in many studies as reliable biomarkers of AD (Craig-Schapiro, et al., 2008) but are infrequently used in the management of individual patients, although they serve differential diagnosis and follow-up in clinical trials. Imaging studies are useful in evaluating the risk of mild cognitive impairment (MCI) progression to AD. In patients with MCI, structural magnetic resonance imaging (MRI) predicted future dementia (Killiany, et al., 2000). PET scanning using the Pittsburgh compound B showed brain A β in MCI patients progressing to AD (Fagan, et al., 2007). However, imaging studies are not ideal for diagnosing AD in younger patients without atrophy detectable by magnetic resonance imaging (MRI) (Schoonenboom, et al., 2008). Moreover, in a follow-up study of A β vaccine trial, MRI brain volume dissociated from cognitive function (Fox, et al., 2005). Low CSF A β and high τ predicted faster progression of cognitive deficits, but the correlation with 2-year future brain atrophy in preclinical subjects was only 0.30 (Fagan, et al., 2009). Consequently, to diagnose the process leading to neurodegeneration in preclinical subjects, novel biomarkers of preclinical AD additive, with or preceding A β , and imaging biomarkers are necessary.

Abnormal innate immune responses to certain aggregated proteins, such as occurs in AD to A β and in amyotrophic lateral sclerosis (ALS) to superoxide dismutase-1 (SOD-1), appear to be the critical immune mechanisms of neurodegenerative diseases. Although inflammation has a long history as the hallmark of AD (Akiyama, et al., 2000), A β phagocytosis has also garnered interest as a diagnostic technique (Fiala, et al., 2007) (Avagyan, et al., 2009).

Immunopathological studies of the AD brain revealed upregulation of complement, cytokines, and acute phase reactants (such as alpha1-antichymotrypsin) in the CNS of AD patients (Akiyama, et al., 2000). Complement activation products C1q, C4b, C3b co-localize with A β plaques and may determine local recruitment and activation of glial cells and degree of removal of A β (Eikelenboom and Veerhuis, 1996). Early neuropathological changes preceding the neurodegenerative changes of AD, i.e. the presence of amyloid- associated factors and clustering of activated cytokine-secreting microglia, together with the role of polymorphism of certain cytokines (IL-1, IL-6, TNF- α) and acute-phase proteins (α 1-antichymotrypsin) as the genetic risk factors for AD, have led to the concept that pro-inflammatory cytokines released by activated microglia are the driving force in AD pathology (Akiyama, et al., 2000).

The innate responses to A β , inflammation and defective A β phagocytosis, are both promising as a basis of new tests for early detection. These tests require traditional and new methods for A β , fluorescence microscopy, flow cytometry, ELISA, and enzyme assays, as well as microarray hybridization, real-time-PCR, high-throughput sequencing, and proteomics techniques to clarify the pathological pathways of AD. In this review, we consider diagnostic testing:: first by cytokine and proteomics fluid biomarkers of AD, second by novel biomarkers related to inflammation, third by those related to phagocytosis, and fourth by transcriptional responses in A β -stressed macrophages.

2. Diagnostic testing by cytokine and proteomics CSF and plasma tests

Although CSF levels of most cytokines cannot be used to diagnose or to differentiate dementias, they might be useful for monitoring therapeutic effects of anti-inflammatory drugs. Moreover, CSF TNF α has been implicated as a modulator of synaptic activity and long term potentiation and was found to be significantly higher in MCI cases, especially in those that progress to AD (Tarkowski, et al., 2003). CSF levels of the anti-inflammatory cytokine transforming growth factor (TGF)- β 1, a regulator of brain responses to inflammation and injury, are consistently found to be increased in AD cases compared to controls (Rota, et al., 2006). MCI cases progressing to AD had lower levels of TGF- β 1 at baseline than controls or nonprogressors, suggesting a propensity to a pro-inflammatory state at the time of progression from MCI to AD (Tarkowski, et al., 2003). Immunohistochemical studies of post mortem brain indicate a

gradual increase in activated microglia and a decrease of COX-2-activated neurons in parallel with the Braak score (i.e. the score for neurofibrillary changes) (Hoozemans et al, 2005).

Other potential biomarkers may be acute phase proteins or other amyloid associated proteins, markers of lipid peroxidation and early markers for neurodegeneration and regeneration. A number of amyloid associated factors (cytokines, chemokines, complement factors and acute phase proteins) have been found to be altered in the CSF in some studies. C-reactive protein (CRP) and IL-6 were significantly higher in the CSF of MCI patients compared to AD patients ($p < 0.01$), suggesting that inflammatory processes are already ongoing and detectable in CSF, even before A β decline and τ increases in CSF, which was especially noticeable in those MCI cases with a low risk AD profile (A β 42 >495 pg/ml and total τ < 356 pg/ml or P- τ < 54 pg/ml) (Schuitemaker, et al., 2008) (Fig. 1). Serum amyloid P component (SAP) may be another biomarker of progression to AD. SAP is mainly produced in the liver, but also locally in the brain. SAP binds to amyloid and may protect A β from proteolysis. In addition, SAP may be directly neurotoxic. In a recent cross-sectional study, no differences in CSF SAP levels between controls, AD patients and MCI cases were observed. However, after a 2-3 year follow-up, the MCI patients who progressed to AD had significantly lower CSF SAP levels in comparison to non-progressors (median 13 and 20 mg/L; $p < 0.05$). After correction for sex, age and MMSE, low SAP levels were associated with a two-fold increased risk of progression to AD (Hazard ratio = 2.2 (95% CI 0.9 to 4.6)), which suggests that SAP may serve as a CSF biomarker to distinguish progressors from non-progressors (Verwey, et al., 2008). We speculate that the non-progressor MCI cases have normal CSF SAP levels, because of low amounts of fibrillar A β deposits and “normal” SAP synthesis, whereas the progressor MCI cases already have fibrillar A β deposits that bind SAP and consequently less SAP draining to CSF. After MCI progression to AD, activated glial cells produce cytokines and consequently acute phase proteins including SAP and, consequently, SAP production increases and, despite consumption by binding to fibrillar amyloid, CSF SAP levels are restored. A dicarboxylic acid, pyrrolidone ring-containing compound (CPHPC), was shown to cross-link SAP pentamers leading to their clearance from the circulation and diminished amyloid deposits in systemic amyloidosis patients. In a recent study, CPHPC was shown to lower plasma as well as CSF levels of SAP in AD patients (Kolstoe, et al., 2009), opening new avenues for treatment.

In the post-mortem brain, increased cyclooxygenase-2 (COX-2), cell cycle protein expression and activation of the unfolded response were detected in mid-temporal neurons in Braak stage III -IV in association with diffuse (low-fibrillar) A β plaques. Neuronal COX-2 and cell cycle protein expression again decreased in stages V and VI, when profound τ -related neurofibrillary changes were observed (Hoozemans, et al., 2005). Thus, the levels of inflammatory cytokines, including prostaglandin E2 (PGE2), appear to be increased in early, compared to late AD stages. F2-isoprostanes, the end-products of lipid peroxidation, were augmented in the CSF of AD cases and increased F2-isoprostanone levels predicted progression from MCI to AD, and possibly from pre-clinical state to MCI (de Leon, et al., 2007).

Transcriptional microarray studies of AD hippocampal tissues showed that the main alterations in early AD cases included decreases in neurotrophic support and activation of apoptotic and neuroinflammatory signaling (Colangelo, et al., 2002). To identify new biomarkers in the serum or CSF, global pattern of proteins is analyzed using either 2-D gel electrophoresis followed by matrix-assisted laser desorption/ionization- time of flight (MALDI TOF) or protein chip arrays on which bound proteins are identified by surface enhanced laser desorption ionization (SELDI)- time-of-flight (TOF)- mass spectrometry (MS). A multiplex quantitative proteomics method identified a panel of unique AD vs. Parkinson disease vs. dementia with Lewy body (DLB) protein markers in the CSF (Abdi, et al., 2006). In another study, complement factor H (CFH) precursor and alpha-2-macroglobulin were identified as significantly different in patients compared to age-matched controls (Hye, et al., 2006). Using the SELDI TOF approach,

15 biomarkers, including cystatin C, N-terminally truncated cystatin C, A β 1-40, and complement anaphylatoxin C3a desArg, were selected that, combined with t and A β 1-42 ELISA data, could distinguish patients with stable MCI from patients with MCI progressing to AD (Simonsen, et al., 2007). Another study produced a panel of signaling plasma proteins related to hematopoiesis, immune responses, apoptosis and neuronal support, which was predictive of progression of MCI to AD and confirmatory of AD diagnosis (Ray, et al., 2007). Taken together, a number of factors that are either up or down regulated or exist in certain isoforms or splice products have been reported, but these findings seem to vary in different stages of AD (for a comprehensive overview, see (Zetterberg, et al., 2008)).

PBMC's of AD patients have crucial transcriptional defects of *MGAT-III* and other genes detected by microarray hybridization (Fiala, et al., 2007). In addition, we have recently observed in AD PBMC's defective expression of another glycan transferase *MGAT-II* using Affymetrix manufactured custom oligo glycan array (Fiala and Head, unpublished data). We have also shown by 2D-DIGE electrophoresis of differentially-labeled proteins of AD PBMC's before and after exposure to A β differences in the expression of 8 proteins (Fiala, Avliyakov and Haykinson, unpublished observations) (Fig. 2). These proteomics techniques will be helpful to clarify the pathways responsible for defective phagocytosis in AD patients.

3. A β clearance from the brain and tests of A β phagocytosis

The immunopathology of neurodegenerative diseases involves molecular perturbations embroiling both the nervous and immune systems dealing with disposal of extra- and intracellular waste-products with propensity to aggregation, such as A β in AD and superoxide dismutase-1 (SOD-1) in ALS. In addition, autophagic degradation of intracellular components, such as mitochondria, via the lysosomal pathway, is abnormal in AD neurons (Boland, et al., 2008). The characteristic pathology of AD, accumulation of A β , may be initially caused by defects in physiological mechanisms of clearance and degradation of A β in the brain. Physiological clearance of A β may occur through drainage to perivascular spaces, or actively through uptake and processing of the A β by astrocytes, microglia or monocyte-derived macrophages. Fibrillar deposits are probably accessible only to macrophages.

3.1 Physiological clearance of A β

Studies in mouse models showed that physiological clearance of A β across the blood-brain barrier (BBB) depends upon A β binding to low-density lipoprotein receptor related protein-1 (LRP1) and receptor for advanced glycation end products (RAGE). In the plasma of AD patients, LRP1 is decreased (Deane, et al., 2009). In the AD brain, RAGE, CD36, and low-density lipoprotein receptor (LDLR) are associated with cerebral amyloid angiopathy. LRP-1 and LDLR are involved in A β internalization and A β -mediated cell death of cerebral perivascular cells (Wilhelmus, et al., 2007). In addition, megalin/low-density lipoprotein receptor-related protein-2 (LRP2) mediates insulin-like growth factor-1 -induced clearance of A β through the choroid plexus (Carro, et al., 2005). Physiological degradation of A β involves degradation by neprilysin (NEP), insulin-degrading enzyme, endothelin-converting enzyme, plasmin, and cathepsin B (Morelli, et al., 2005). Differences in matrix metalloproteinase-9 (MMP-9) activity contribute to the apo E isoform-specific differences in A β degradation by macrophages (Zhao, et al., 2009).

The physiological clearance is clearly not sufficient in the AD brain, where A β assemblies accumulate both extra- and intraneuronally: Oligomeric A β accumulates in neurons and fibrillar A β in plaques and congophilic vessels (Zaghi, et al., 2009). APP transgenic animal models do not reproduce the immune transcriptional defects of human patients (Fiala, et al., 2007). Thus the pathways of immune clearance in human brain must be assembled piecemeal

from immunochemical studies of post-mortem brains and in vitro studies of astrocytes, microglia and blood macrophages.

3.2 Role of astrocytes

In the human brain, A β accumulates inside astrocytes in areas remote from fibrillar A β deposits, suggesting that astrocytes can ingest A β at early stages of A β accumulation. Primary adult as well as fetal astrocytes bind and ingest fluorescent A β when examined by flow cytometry and microscopy (Nielsen, et al., 2009). No significant difference in A β 1-42 uptake between AD and non-AD astrocytes and no influence of ApoE genotype on A β 1-42 uptake were observed. Because A β containing astrocytes were only found in areas with nonfibrillar A β , it is possible that astrocytes may be the first in line to clear A β from the interstitial space, but the A β peptide readily aggregates and forms fibrils, which may escape the reach of astrocytes. Various amyloid associated proteins, such as SAP, complement factors, apolipoproteins and various glycosaminoglycans, may enhance the aggregation process.

3.3 Role of microglia

Microglia may be the second in line to clear the A β deposits, but probably are also unable to ingest large deposits because of their poor phagocytic function, as judged from flow cytometric testing of the uptake of fluorescently labeled A β 1-42 by adult human microglia (Familian, et al., 2007). Since neuroinflammation in AD is characterized by both degeneration and regeneration that occurs in a spatial and temporal specific pattern (Hoozemans, et al., 2006), microglia may change their activation state in time. In comparison to monocyte-derived macrophages, microglia from adult human brain were found to be less eager to ingest fluorescent labeled A β 1-42 in vitro but to produce higher levels of the cytokines TNF- and IL-6 in response to A β 1-42 in combination with complement factor C1q and SAP (Familian, et al., 2007) (Fig. 1). The CD68- immunoreactive, ramified and also the amoeboid, activated microglia seen associated with fibrillar A β plaques in post mortem temporal cortex specimens from AD patients were COX-2 negative, CD14-dim (25% isolated microglia (~10 days after isolation) were positive by flow cytometry) but to some extent exhibiting Toll-like receptor 4 (TLR-4) immunopositive vesicles.

Increased TLR4 immunostaining can be detected in glial cells surrounding plaques in AD patients (Walter et al 2007). Although a fully functional receptor complex of TLR4, MD-2 and CD14 is required for full activation of microglia, TLR4 has been found to mediate mouse microglia and human monocyte activation by aggregated A β , resulting in release of inflammatory products and in microglial neurotoxicity (Walter et al 2007).

Immune cells in the AD brain appear to exhibit a hybrid activation state that includes characteristics of classical (inflammatory) and alternative (repair and extracellular matrix remodeling) activation (Colton, et al., 2006). Repair processes mediated by alternative activation genes, however, can also lead to fibrosis and in AD brain possibly to activation of astrocytes with concomitant production of factors that enhance encapsulation of the fibrillar A β deposits. This would, in turn, lead to a subsiding of the inflammatory response and to microglia returning to a resting state, a phenomenon observed in later Braak stages (Veerhuis, et al., 2005)

3.4 Role of macrophages

The lion share of A β clean-up is probably reserved for blood-derived macrophages, which are effective phagocytes able to surround and ingest even large chunks of A β when tested in vitro. However, only macrophages of control subjects are fully functional, whereas macrophages or monocytes of AD patients are poorly phagocytic for A β , which is retained on cell surface, and are susceptible to apoptosis (Avagyan, et al., 2009) (Zaghi, et al., 2009). Transcription of Toll-

like receptors (TLR's) 1-10 is decreased in macrophages of AD patients compared to macrophages of control subjects (Fiala, et al., 2007).

Immunohistochemical examination of AD brains with Braak stages III to V showed increased numbers of macrophages in perivascular spaces and the neuropil compared to age-matched control subjects (Fiala, et al., 2002). In addition, large macrophages distinct from microglia by their large size were noted to migrate across the vessel wall into the neuropil and invade and partially clear neuritic plaques. These CD68-positive macrophages included COX-2-positive (up to 56%) and iNOS-positive (40%) subsets. In the experience of the authors, only perivascular macrophages were CD14-positive (Fiala, et al., 2001) (Veerhuis, unpublished data). In contrast, microglia in the parenchyma (even microglia in neuritic plaques) were, only occasionally, faintly CD14-immunoreactive (Veerhuis, unpublished data). In vitro, 80% of monocyte-derived macrophages isolated from blood of healthy donors were positive by flow cytometry (Veerhuis, unpublished data). Since AD patients have an increased percentage of CD14+/CD69+ monocytes in peripheral blood (Kusdra, et al., 2000), the expression of CD14 on macrophages in the brain may depend upon the stage of the disease as well as the process of monocyte immigration. Ramified microglia were on the periphery of neuritic plaques and appeared A β -negative (Fiala, et al., 2002). Brain microglia, astrocytes and neurons secrete chemokines, which are responsible for attracting monocytes to migrate across the blood-brain barrier from microvessels into the neuropil (Fiala, et al., 2005).

3.5 Flow cytometric test of A β phagocytosis

Phagocytosis of A β can be tested using in vitro differentiated macrophages exposed to fluorescent A β or using a "tissue assay" with monocytes incubated on AD brain tissues in vitro for 2-4 days (monocytes are labeled using CellTracker (Invitrogen) or detected by immunofluorescent staining with anti-CD68) (Fiala, et al., 2007). Macrophages and monocytes of AD patients are generally poorly phagocytic for A β , which is retained on cell surface, whereas control subjects' macrophages robustly phagocytize Ab without undergoing apoptosis. In the AD brain, a subset of endogenous macrophages is phagocytic for oligomeric Ab and probably migrates to congophilic vessels, but these macrophages suffer apoptosis spilling oligomeric Ab into congophilic deposits (Zaghi, et al., 2009). In the tissue assay, monocytes of control subjects up load Ab from neurons and degrade it, whereas monocyte/ macrophages of AD patients suffer apoptosis.

Based on these considerations of the role of macrophages in clearance of A β , we have developed a flow cytometric assay of A β phagocytosis using peripheral blood monocytes. The assay was tested in a pilot study of 18 AD patients (mean age 77.4 years) and 14 control subjects, active professors and businessmen (mean age 74.2 years) (Avagyan, et al., 2009). The test was positive, i.e. showing defective phagocytosis < 400 MFI Units in 94% patients, whereas 0% active age-matched control subjects had defective phagocytosis. However, 75 % caregivers were positive in this assay. The caregivers are at risk of AD due to exposure to a high stress of care giving and lack of mental and physical exercise. Future prospective studies are necessary to determine the value of the test for predicting the risk of dementia, which was suggested in a small sample of MCI patients by 60% positive results. The test may be useful for follow-up of the immune status of patients with AD or risk of AD on various immunomodulating therapies, such as curcuminoids (Fiala, et al., 2007) or vitamin D3 recently shown to improve phagocytosis of A β in AD macrophages in vitro (Masoumi, et al., 2009).

4. Transcriptome of macrophages stressed by A β

To understand the molecular basis of AD patients' macrophage defects, we tested the transcriptome of human PBMC's stressed with A β using the Operon Human Genome Oligo Set Version 3.0 array. Thirty five genes were up regulated >2.2 fold in normal PBMC's stressed

with A β compared to AD PBMC's stressed with A β , including the critical gene, *MGAT-III*, which was up regulated 327 fold. The product of *MGAT-III* may have a critical role in phagocytosis since transfection of normal monocytes and macrophages by *MGAT-III* siRNA blocked A β phagocytosis. In addition, all *TLR*'s were downregulated in PBMC's of AD patients during phagocytosis (Fiala, et al., 2007). In microglial cell lines and transgenic mice, individual *TLR*'s have been investigated for their role in inflammation (*TLR4*), A β clearance (*TLR2*) and neuronal apoptosis. However, in AD patients' monocytes, no single gene defects but a web of transcriptional (Fiala, et al., 2007) and signaling (Fiala, unpublished observation) defects appear to lead to poor differentiation and maturation of macrophages, decreased phagocytosis and degradation, and increased inflammation.

5. Conclusions

A β /P- τ -based CSF tests and MR and PET imaging tests are reliable tests of A β pathology in the brain. Novel tests based on phagocytosis and/or inflammation may be useful in predicting future risk of AD, but the power of these tests will need to be examined in prospective studies. Positive prediction of the risk of AD in aged retired subjects is a daunting task. The collusion between the pathways of aging per se and those of AD renders a difficult comparison of AD patients to age-matched controls from general population (especially in stressed caregivers). However, a comparison of AD patients with active age-matched professors and businessmen by the flow cytometric test in our pilot study showed significant differences with a clear break-point between patients and controls. The flow cytometric test is based on A β pathways specific for AD and might have a high specificity for AD even in very old age.

Peripheral blood-derived macrophages from patients and control subjects have distinct properties *in vitro* and *in vivo*. AD macrophages show increased propensity to apoptosis and decreased ability of A β phagocytosis *in vitro* and *in vivo*, whereas control macrophages are fully functional. The underlying transcriptional pathologies of AD patients are complex and will need to be resolved by molecular techniques but already provide diagnostic biomarkers for AD and future risk of AD. These biomarkers may be useful for the follow-up of individual patients on various immunomodulating therapies.

Acknowledgments

The development of a flow cytometric test has been supported by MPBiomedicals, LLC (M.F.). Glycan array hybridization was supported by a grant from National Institute of General Medical Sciences GM62116 to the Consortium for Functional Glycomics. 2D-DIGE electrophoresis was performed by the Functional Proteomics and Imaging Laboratory of the UCLA Biological Chemistry Department. Recent work on adult human glial cells discussed in this review was financially supported by Stichting Dioraphte (R.V.) and by the Internationale Stichting Alzheimer Onderzoek (ISAO; grant 06517 to R.V.)

References

1. Abdi F, Quinn JF, Jankovic J, McIntosh M, Leverenz JB, Peskind E, Nixon R, Nutt J, Chung K, Zabetian C, Samii A, Lin M, Hattan S, Pan C, Wang Y, Jin J, Zhu D, Li GJ, Liu Y, Waichunas D, Montine TJ, Zhang J. Detection of biomarkers with a multiplex quantitative proteomic platform in cerebrospinal fluid of patients with neurodegenerative disorders. *J Alzheimers Dis* 2006;9:293–348. [PubMed: 16914840]
2. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. Inflammation and Alzheimer's disease. *Neurobiol Aging* 2000;21:383–421. [PubMed: 10858586]

3. Avagyan H, Goldenson B, Tse E, Masoumi A, Porter V, Wiedau-Pazos M, Sayre J, Ong R, Mahanian M, Koo P, Bae S, Micic M, Liu PT, Rosenthal MJ, Fiala M. Immune blood biomarkers of Alzheimer disease patients. *J Neuroimmunol* 2009;210:67–72. [PubMed: 19329192]
4. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurol* 2003;2:605–613. [PubMed: 14505582]
5. Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, Nixon RA. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. *J Neurosci* 2008;28:6926–6937. [PubMed: 18596167]
6. Carro E, Spuch C, Trejo JL, Antequera D, Torres-Aleman I. Choroid plexus megalin is involved in neuroprotection by serum insulin-like growth factor I. *J Neurosci* 2005;25:10884–10893. [PubMed: 16306401]
7. Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. *J Neurosci Res* 2002;70:462–473. [PubMed: 12391607]
8. Colton CA, Mott RT, Sharpe H, Xu Q, Van Nostrand WE, Vitek MP. Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. *J Neuroinflammation* 2006;3:27. [PubMed: 17005052]
9. Craig-Schapiro R, Fagan AM, Holtzman DM. Biomarkers of Alzheimer's disease. *Neurobiol Dis.* 2008
10. de Leon MJ, Mosconi L, Li J, De Santi S, Yao Y, Tsui WH, Pirraglia E, Rich K, Javier E, Brys M, Glodzik L, Switalski R, Saint Louis LA, Pratico D. Longitudinal CSF isoprostane and MRI atrophy in the progression to AD. *J Neurol* 2007;254:1666–1675. [PubMed: 17994313]
11. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol Disord Drug Targets* 2009;8:16–30. [PubMed: 19275634]
12. Eikelenboom P, Veerhuis R. The role of complement and activated microglia in the pathogenesis of Alzheimer's disease. *Neurobiol Aging* 1996;17:673–680. [PubMed: 8892339]
13. Fagan AM, Head D, Shah AR, Marcus D, Mintun M, Morris JC, Holtzman DM. Decreased cerebrospinal fluid Aβ(42) correlates with brain atrophy in cognitively normal elderly. *Ann Neurol* 2009;65:176–183. [PubMed: 19260027]
14. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/β-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol* 2007;64:343–349. [PubMed: 17210801]
15. Familian A, Eikelenboom P, Veerhuis R. Minocycline does not affect amyloid beta phagocytosis by human microglial cells. *Neurosci Lett* 2007;416:87–91. [PubMed: 17317005]
16. Fiala M, Lin J, Ringman J, Kermani-Arab V, Tsao G, Patel A, Lossinsky AS, Graves MC, Gustavson A, Sayre J, Sofroni E, Suarez T, Chiappelli F, Bernard G. Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *J Alzheimers Dis* 2005;7:221–232. discussion 255–262. [PubMed: 16006665]
17. Fiala M, Liu NQ, Reddy S, Graves MC. Macrophages infiltrate brain and express COX-2 and iNOS in Alzheimer's disease and AIDS. *Alzheimer's Reports* 2001;4:1–7.
18. Fiala M, Liu PT, Espinosa-Jeffrey A, Rosenthal MJ, Bernard G, Ringman JM, Sayre J, Zhang L, Zaghi J, Dejbakhsh S, Chiang B, Hui J, Mahanian M, Baghaee A, Hong P, Cashman J. Innate immunity and transcription of MGAT-III and Toll-like receptors in Alzheimer's disease patients are improved by bisdemethoxycurcumin. *Proc Natl Acad Sci U S A* 2007;104:12849–12854. [PubMed: 17652175]
19. Fiala M, Liu QN, Sayre J, Pop V, Brahmandam V, Graves MC, Vinters HV. Cyclooxygenase-2-positive macrophages infiltrate the Alzheimer's disease brain and damage the blood-brain barrier. *Eur J Clin Invest* 2002;32:360–371. [PubMed: 12027877]
20. Fox NC, Black RS, Gilman S, Rossor MN, Griffith SG, Jenkins L, Koller M. Effects of Aβ immunization (AN1792) on MRI measures of cerebral volume in Alzheimer disease. *Neurology* 2005;64:1563–1572. [PubMed: 15883317]
21. Hoozemans JJ, van Haastert ES, Veerhuis R, Arendt T, Scheper W, Eikelenboom P, Rozemuller AJ. Maximal COX-2 and ppRb expression in neurons occurs during early Braak stages prior to the

- maximal activation of astrocytes and microglia in Alzheimer's disease. *J Neuroinflammation* 2005;2:27. [PubMed: 16300681]
22. Hoozemans JJ, Veerhuis R, Rozemuller JM, Eikelenboom P. Neuroinflammation and regeneration in the early stages of Alzheimer's disease pathology. *Int J Dev Neurosci* 2006;24:157–165. [PubMed: 16384684]
 23. Hye A, Lynham S, Thambisetty M, Causevic M, Campbell J, Byers HL, Hooper C, Rijdsdijk F, Tabrizi SJ, Banner S, Shaw CE, Foy C, Poppe M, Archer N, Hamilton G, Powell J, Brown RG, Sham P, Ward M, Lovestone S. Proteome-based plasma biomarkers for Alzheimer's disease. *Brain* 2006;129:3042–3050. [PubMed: 17071923]
 24. Killiany RJ, Gomez-Isla T, Moss M, Kikinis R, Sandor T, Jolesz F, Tanzi R, Jones K, Hyman BT, Albert MS. Use of structural magnetic resonance imaging to predict who will get Alzheimer's disease. *Ann Neurol* 2000;47:430–439. [PubMed: 10762153]
 25. Kolstoe SE, Ridha BH, Bellotti V, Wang N, Robinson CV, Crutch SJ, Keir G, Kukkastenvehmas R, Gallimore JR, Hutchinson WL, Hawkins PN, Wood SP, Rossor MN, Pepys MB. Molecular dissection of Alzheimer's disease neuropathology by depletion of serum amyloid P component. *Proc Natl Acad Sci U S A* 2009;106:7619–7623. [PubMed: 19372378]
 26. Kusdra L, Rempel H, Yaffe K, Pulliam L. Elevation of CD69+ monocyte/macrophages in patients with Alzheimer's disease [In Process Citation]. *Immunobiology* 2000;202:26–33. [PubMed: 10879686]
 27. Masoumi A, Goldenson B, Ghirmai S, Avagyan H, Zaghi J, Abel K, Zheng X, Espinosa-Jeffrey A, Mahanian M, Liu PT, Hewison M, Mizwicki M, Cashman J, Fiala M. 1alpha,25-dihydroxyvitamin D3 Interacts with Curcuminoids to Stimulate Amyloid-beta Clearance by Macrophages of Alzheimer's Disease Patients. *J Alzheimers Dis.* 2009
 28. Morelli L, Bulloj A, Leal MC, Castano EM. Amyloid beta degradation: a challenging task for brain peptidases. *Subcell Biochem* 2005;38:129–145. [PubMed: 15709476]
 29. Nielsen HM, Veerhuis R, Holmqvist B, Janciauskiene S. Binding and uptake of A beta1-42 by primary human astrocytes in vitro. *Glia* 2009;57:978–988. [PubMed: 19062178]
 30. Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, Friedman LF, Galasko DR, Jutel M, Karydas A, Kaye JA, Leszek J, Miller BL, Minthon L, Quinn JF, Rabinovici GD, Robinson WH, Sabbagh MN, So YT, Sparks DL, Tabaton M, Tinklenberg J, Yesavage JA, Tibshirani R, Wyss-Coray T. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Med* 2007;13:1359–1362. [PubMed: 17934472]
 31. Riley KP, Snowden DA, Desrosiers MF, Markesbery WR. Early life linguistic ability, late life cognitive function, and neuropathology: findings from the Nun Study. *Neurobiol Aging* 2005;26:341–347. [PubMed: 15639312]
 32. Rota E, Bellone G, Rocca P, Bergamasco B, Emanuelli G, Ferrero P. Increased intrathecal TGF-beta1, but not IL-12, IFN-gamma and IL-10 levels in Alzheimer's disease patients. *Neurol Sci* 2006;27:33–39. [PubMed: 16688597]
 33. Schoonenboom NS, van der Flier WM, Blankenstein MA, Bouwman FH, Van Kamp GJ, Barkhof F, Scheltens P. CSF and MRI markers independently contribute to the diagnosis of Alzheimer's disease. *Neurobiol Aging* 2008;29:669–675. [PubMed: 17208336]
 34. Schuitemaker A, Dik MG, Veerhuis R, Scheltens P, Schoonenboom NS, Hack CE, Blankenstein MA, Jonker C. Inflammatory markers in AD and MCI patients with different biomarker profiles. *Neurobiol Aging*. 2008 doi:10.1016/j.neurobiolaging.2008.01.014.
 35. Simonsen AH, McGuire J, Hansson O, Zetterberg H, Podust VN, Davies HA, Waldemar G, Minthon L, Blennow K. Novel panel of cerebrospinal fluid biomarkers for the prediction of progression to Alzheimer dementia in patients with mild cognitive impairment. *Arch Neurol* 2007;64:366–370. [PubMed: 17353378]
 36. Sunderland T, Linker G, Mirza N, Putnam KT, Friedman DL, Kimmel LH, Bergeson J, Manetti GJ, Zimmermann M, Tang B, Bartko JJ, Cohen RM. Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. *JAMA* 2003;289:2094–2103. [PubMed: 12709467]

37. Tarkowski E, Andreasen N, Tarkowski A, Blennow K. Intrathecal inflammation precedes development of Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2003;74:1200–1205. [PubMed: 12933918]
38. Veerhuis R, Boshuizen RS, Familian A. Amyloid associated proteins in Alzheimer's and prion disease. *Curr Drug Targets CNS Neurol Disord* 2005;4:235–248. [PubMed: 15975027]
39. Verwey NA, Schuitemaker A, van der Flier WM, Mulder SD, Mulder C, Hack CE, Scheltens P, Blankenstein MA, Veerhuis R. Serum amyloid p component as a biomarker in mild cognitive impairment and Alzheimer's disease. *Dement Geriatr Cogn Disord* 2008;26:522–527. [PubMed: 19052452]
40. Wilhelmus MM, Otte-Holler I, van Triel JJ, Veerhuis R, Maat-Schieman ML, Bu G, de Waal RM, Verbeek MM. Lipoprotein receptor-related protein-1 mediates amyloid-beta-mediated cell death of cerebrovascular cells. *Am J Pathol* 2007;171:1989–1999. [PubMed: 18055545]
41. Zaghi J, Goldenson B, Inayathullah M, Lossinsky AS, Masoumi A, Avagyan H, Mahanian M, Bernas M, Weinand M, Rosenthal MJ, Espinosa-Jeffrey A, de Vellis J, Teplow DB, Fiala M. Alzheimer disease macrophages shuttle amyloid-beta from neurons to vessels, contributing to amyloid angiopathy. *Acta Neuropathol* 2009;117:111–124. [PubMed: 19139910]
42. Zetterberg H, Ruetschi U, Portelius E, Brinkmalm G, Andreasson U, Blennow K, Brinkmalm A. Clinical proteomics in neurodegenerative disorders. *Acta Neurol Scand* 2008;118:1–11. [PubMed: 18279484]
43. Zhao L, Lin S, Bales KR, Gelfanova V, Koger D, Delong C, Hale J, Liu F, Hunter JM, Paul SM. Macrophage-mediated degradation of beta-amyloid via an apolipoprotein E isoform-dependent mechanism. *J Neurosci* 2009;29:3603–3612. [PubMed: 19295164]

Figure 1. Diagram of putative events associated with extracellular Aβ deposition and clearance (Adapted from Veerhuis et al, 2005)

(1) Monomeric and small oligomeric Aβ are prevented from neurotoxic action through astrocytic uptake; (2) Amyloid associated factors (AAF's) enhance formation of Aβ aggregates indigestible by astrocytes; (3) Microglia move in to clear the aggregated Aβ; (4) AAF's enhance Aβ fibrillization; (5) In late stage, AAF's lead to Aβ deposit encapsulation and sequestration, and subsidence of the inflammatory response; (6) In intermediate stage, AAF's activate microglia to secrete neurotoxic and inflammatory agents, which stimulate synthesis of APP and AAF's, and are chemotactic; (7) Monocyte/macrophages ingest larger Aβ aggregates but eventually die and contribute to vascular amyloid formation.

Figure 2. Differentially-expressed proteins in A β -stimulated or not stimulated PBMC's of an AD patient
Proteins from PBMC's cultured without A β were labeled with Cy3 (panel A), those cultured with A β were labeled with Cy5 (panel B), and both were examined by 2D-DIGE electrophoresis. Protein spots showing significant changes of more than 2.5-fold are shown. Proteins showing a decreased expression after incubation with A β are labeled red and those showing an increased expression after incubation are labeled blue. There were 8 spots showing at least 2.5-fold change in protein expression, five of them showing an increased expression upon A β treatment and 3 spots (red) showing a decreased expression (panels C and D). Although the proteins have not yet been identified, the observed characteristic spot pattern may be due to a shift in post-translational modification of these proteins.

Fig. 1 Phagocytosis of amyloid-beta by patients and control subjects

Alzheimer disease patient (Age 73 years)

Control subject (Age 73 years)

Figure 3. Flow cytometric test results in AD patients and control subjects

PBMC's were incubated overnight with FAM-A β and, after staining with anti CD14-PE, were tested by flow cytometry as described (Avagyan, et al., 2009). Monocytes of control subject bind more FAM-A β compared to patients.