

LETTERS

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IL-6 release by LPS-stimulated peripheral blood mononuclear cells as a potential biomarker in Alzheimer's disease

There is increasing evidence for the role of activated microglia in the neurotoxic pathways of Alzheimer's disease (AD). Amyloid- β_{1-42} is a potent activator of microglia (Benveniste *et al.*, 2001), causing them to release pro-inflammatory cytokines including IL-6, IL-1 β , TNF- α among others. A peripheral blood marker reflecting CNS inflammatory processes would be useful for treatment development, but the search for such an *in vivo* marker has been elusive. Peripheral blood and CSF levels of pro-inflammatory cytokines do not consistently differ in AD and controls (reviewed in Rosenberg, 2005). Both microglia and peripheral blood mononuclear cells (PBMCs) release pro-inflammatory cytokines when exposed to immunologic stimuli including lipopolysaccharide (LPS) and phytohemagglutinin (PHA). There are recent data suggesting that PBMCs from AD patients release more cytokines in this paradigm than normal controls (Reale *et al.*, 2004).

We sought to replicate these studies by exposing PBMCs both to the non-specific microglial activator LPS and to A- β_{1-42} as an AD-specific microglial activator. In addition, given the potential links between inflammation, AD and mood symptoms (Benveniste *et al.*, 2001; Miller and Raison, 2006), we examined associations between this biomarker and neuropsychiatric symptoms as well as performance on standard cognitive tests. Our hypotheses were that (1) PBMCs from AD participants would release more IL-6 with LPS stimulation than PBMCs from control participants; and (2) A- β_{1-42} exposure would magnify IL-6 release disproportionately for AD cases as compared to controls.

Study participants were enrolled and had been characterized in the Johns Hopkins University Alzheimer's Disease Research Center. AD was diagnosed using the criteria proposed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA criteria), while control participants had no cognitive diagnosis. Diagnoses were made by an interdisciplinary research treatment team including geriatric psychiatrists, neurologists, neuropsycho-

logists and nurses. All AD participants had a knowledgeable informant for clinical measures. The participant or legally authorized representative signed informed consent, and the studies were approved by the Johns Hopkins Institutional Review Board. Clinical Measures included the Clinical Dementia Rating Scale (CDR), Mini-mental State Examination (MMSE), category verbal fluency, and the Neuropsychiatric Inventory (NPI). For each subject, 60 cc of blood was drawn into heparinized tubes and PBMCs purified by standard Ficoll gradient methods, then cryopreserved. PBMCs were subsequently thawed and exposed to T-cell medium or to LPS in concentrations of 20 or 100 ng/ml; similar contrasts were made between exposure to T-cell medium vs. A- β_{1-42} 20 μ g/ml. Concentrations were chosen by prior experiments and extrapolations from the literature. Supernatants were taken at 48 hours and IL-6 measured by standard ELISA kit (R&D Biosystems). The primary outcome was the ratio of IL-6 release with LPS exposure to IL-6 release without LPS exposure ("IL-6 release ratio"). Paired Students' t-tests were used to assess individual differences in IL-6 release ratio between AD and control participants. Linear regression was used to correlate the IL-6 release ratio with clinical measures. Statistical significance was set *a priori* at $\alpha = 0.05$. Data analysis was performed with Stata 8 statistical software (College Station, TX).

Age-gender matched participants with AD ($n = 5$, mean age 78 \pm 2.0) and cognitively normal controls ($n = 5$, mean age 79 \pm 1.8) were enrolled. One pair was female (20%). At an LPS concentration of 20 ng/ml with no A- β_{1-42} , the mean IL-6 release ratio was 53 (SD 26) for controls and 61 (SD 15) for AD ($p = 0.75$, Student's t); the corresponding ratios with A- β_{1-42} 20 μ g/ml were 60 (SD 29) for controls and 71 (SD 12) for AD ($p = 0.75$). At an LPS concentration of 100 ng/ml with no A- β_{1-42} , the IL-6 release ratio was 45 (SD 22) for controls and 94 (SD 32) for AD ($p = 0.07$); the corresponding ratios with A- β_{1-42} 20 μ g/ml were 59 (SD 30) for controls and 101 (SD 12) for AD ($p = 0.12$). The ratio of IL-6 release ratio with A- β_{1-42} 20 μ g/ml to no A- β_{1-42} exposure was 2.09 (SD 1.05) for AD vs. 1.1 (SD 0.12) for controls at LPS 20 ng/ml ($p = 0.43$); similar results were seen with LPS 100 ng/ml (data not shown). There were significant correlations between IL-6 release ratio and both cognitive and NPS measures. IL-6 release ratio was significantly correlated with *worse* animal category fluency

($p = 0.03$, $r^2 = 0.49$) and *higher* NPI total scores ($p = 0.01$, $r^2 = 0.63$) (see Figures S1A and S1B, respectively; available as supplementary material attached to the electronic version of this letter at www.journals.cambridge.org/jid_IPG). Similarly, there were *trend* level correlations of IL-6 release with worse ratings on the CDR ($p = 0.08$, $r^2 = 0.33$) and MMSE ($p = 0.05$, $r^2 = 0.39$).

In this pilot project, we sought to replicate prior findings suggesting that IL-6 release from LPS-stimulated PBMCs is higher in AD than cognitively normal controls. We found increased IL-6 release ratios in AD, with trend level significance at the higher LPS concentration only. This is not surprising given the small sample; since the results are in the predicted direction they offer support for hypothesis 1. We sought to extend prior results by adding an AD-specific stimulus (A- β_{1-42}), in an attempt to more closely model the neuroinflammatory processes of AD. Modest support was seen for hypothesis 2 with A- β_{1-42} increasing the IL-6 release ratio by a factor of 2 in AD patients but not controls, although this was not statistically significant; these results are in the direction predicted by hypothesis 2.

Additionally we found a significant trend correlation between IL-6 release ratio and several clinical variables, including verbal category fluency (animals), NPI, CDR and MMSE. A higher IL-6 release ratio was associated with worse illness severity (higher NPI total and CDR, lower MMSE and category fluency). This suggests that the IL-6 release ratio may have value as a marker of AD disease severity, especially severity of *neuropsychiatric symptoms* (NPS). The latter appears to be a unique finding, and may reflect the involvement of inflammatory mechanisms (peripheral and central) in the development of NPS.

This study is limited by small sample size, assessment of only one candidate cytokine, and limited capacity to assess multiple reaction conditions. The strengths of the study include

a well-characterized cohort of AD and control participants and the extension of prior results to neuropsychiatric and functional as well as cognitive findings.

These preliminary results suggest that IL-6 release from PBMCs may be a useful marker of disease severity and NPS severity in AD, thus meriting further study.

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The relationship between annual predicted future population growth rates and elderly suicide rates

Two paradoxical hypotheses – (i) that countries with low population growth rates or a decline in population growth rate will have high elderly dependency ratios leading to high elderly suicide rates,

and (ii) that countries with high population growth rates will have high elderly suicide rates because of Durkheim's hypothesis that the overall cohort size may influence suicide rates due to competition for scarce resources – were supported by a recent study (Shah, 2008). The relationship between average annual population growth rates and elderly suicide rates was shown to be curvilinear (U-shaped curve) fitting the quadratic equation $y = a + bx + cx^2$ (where y is the elderly suicide rate, x is the average