ADNI Alzheimer's Disease Neuroimaging Initiative

CSF inflammatory proteins Methods - Hu Lab

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Contents

Summary (or Abstract)

Non-beta-amyloid, non-tau (NANT) biomarkers of Alzheimer's disease (AD) can potentially inform disease progression, mechanisms, and endophenotypes. We have focused on CSF markers of neuroinflammation as candidate NANT biomarkers in AD. Epidemiologic, genetic, pathologic, and animal studies have all linked inflammation to AD, and each inflammatory mediator identified by these studies represents a potential NANT biomarker. We and others previously reported altered CSF IL-7 levels in AD through commercial multi-analyte profiling in two independent cohorts,[1, 3] but CSF IL-7 levels were also strongly influenced by gender ($p<0.001$). Follow-up work in assessing CSF IL-7 and fractalkine (CX3CL1, a T-cell chemotractant) levels showed a subgroup of AD patients demonstrating greater T-cell activation. As cytokines and chemokines tend to change in networks rather than in isolation, a more comprehensive evaluation of the CSF cytokine alterations is necessary to accurately inform therapeutic development targeting inflammation in AD. Specifically, we sought to analyze cytokine alterations linked to Type 1 [TH1] vs. Type 2 [TH2] vs. Type 17 [TH17] T-helper cells.

Method and Summary of Results

We analyzed levels of 14 inflammatory proteins in banked CSF samples from ADNI. CSF samples were available from 113 subjects with normal cognition (NC), 185 MCI subjects with CSF consistent with AD (MCI-AD), and 90 subjects with AD dementia. **The first group** of inflammatory proteins involved the IL-7 and IL-10 pathways, given previously known association between IL-7 and AD diagnosis, and between IL-10 and AD progression.[2] Based on current knowledge of IL-7/IL-10 pathways (Fig. 1), we measured CSF levels of pro-inflammatory markers (IL-7, IL-12p40, IFN-γ), anti-inflammatory markers (IL-4, IL-10), and markers associated IL-17 releasing T-helper cells (TH17: TGF-β, IL-6, and IL-21 & IL-22) using commercially available multiplex immunoassays (Millipore Sigma, Burlington, MA) which we have modified for CSF analyte levels.

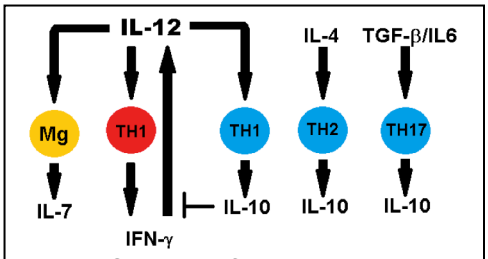

Fig 1. Sources of IL-10 and their effectors. TH1: T-helper type 1; TH2: T-helper type 2; TH17: IL-17 releasing T-helper cells.

A second group of inflammatory proteins related to

TNF-α were measured, including TNF-α and its inhibitory soluble receptors TNF-R1 and TNF-R2.[4] **Finally,** we measured two downstream TNF-α effectors: intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1).

Samples were randomized across twelve 96-well plates for each of the five assays (60 plates total) encompassing fifteen analytes: Tumor Necrosis Factor Receptor 1,2 (TNFR1,2), Transforming Growth Factor 1,2,3 (TGFβ 1,2,3), Interleukin 21 (IL-21), Intercellular Adhesion Molecule 1 (ICAM-1); Vascular Cell Adhesion Molecule 1 (VCAM-1), Tumor Necrosis Factor α (TNFα), Interleukin 6 (IL-6), Interleukin 7 (IL-7), Interleukin 9 (IL-9), Interleukin 10 (IL-10), Interleukin 12 subunit p40 (IL-12 p40), Interferon Gamma-Induced Protein 10 (IP-10). Levels of three cytokines (IL-9, IP-10, and IL-12p40) were not measured in the first three assay plates due to omission of the corresponding antibodies from the kits.

All samples were run in duplicate along with six CSF standards on each plate. Samples were normalized across plates using CSF standard values. Precision of each analyte was calculated using inter-plate coefficient of variation (CV) [Table 1].

	Standard Range (pg/mL)	Median Sample Concentration (pg/mL)	LLOD (MFI)	Median MFI	Inter-plate %CV
TNFR1	12.2 - 50,000	845.44	28.10	1384.13	2.85
TNFR2	12.2 - 50,000	1013.88	16.39	1474.13	3.09
TGFβ1	2.4 - 10,000	102.75	13.58	199.26	8.62
TGFβ2	2.4 - 10,000	153.23	18.08	864.05	7.62
TGFβ3	2.4 - 10,000	2.84	7.07	8.95	7.70
IL-21	5 - 20,000	7.51	23.60	40.59	20.6
IL-6	1.6 - 10,000	3.94	16.25	70.86	6.3
IL-7	1.6 - 10,000	1.05	11.17	15.86	14.68
IL-9	1.6 - 10,000	3.13	34.65	79.06	9.24
IL-10	1.6 - 10,000	5.23	10.02	42.26	16.51
TNFα	1.6 - 10,000	1.70	49.2	53.53	9.38
IP-10	1.6 - 10,000	4990.57	49.86	14701.31	4.83
IL-12 p40	1.6 - 10,000	0.93	23.52	26.01	6.41
ICAM-1	24 - 100,000	322.81	23.98	241.32	9.86
VCAM-1	61 - 250,000	38227.06	15.21	3853.82	10.99

Table 1. Summary of data and precision of each analyte across plates expressed as percent coefficient of variation.

Version Information

This document serves as Version 1.

Dataset Information

This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	Date Submitted
ADNI Data Hu Lab	6 December 2018

References

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