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Immunological biomarkers associated with brain structure and executive function in late-life depression: exploratory pilot study

Stephen F. Smagula, PhD¹, Francis Lotrich, MD PhD¹, Howard J. Aizenstein, MD PhD¹, Breno S. Diniz, MD PhD², Jeffrey Krystek, BS¹, Gregory F. Wu, MD PhD³, Benoit H. Mulsant, MD⁴, Meryl A. Butters, PhD¹, Charles F. Reynolds III, MD^{1,5}, and Eric J. Lenze, MD⁶

¹Department of Psychiatry, Western Psychiatric Institute and Clinic of University of Pittsburgh Medical Center, Pittsburgh, PA, USA

²Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, Houston, TX, USA

³Department of Neurology, Washington University School of Medicine, St Louis, MO, USA

⁴Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada

⁵Department of Behavioral and Community Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

⁶Healthy Mind Lab, Department of Psychiatry, Washington University School of Medicine, St Louis, MO, USA

Abstract

Objective—Several immunological biomarkers are altered in late-life major depressive disorder (LLD). Immunological alterations could contribute to LLD's consequences, but little is known about the relations between specific immunological biomarkers and brain health in LLD. We performed an exploratory pilot study to identify, from several candidates, the specific immunological biomarkers related to important aspects of brain health that are altered in LLD (brain structure and executive function).

Methods—Adults (n=31) were at least 60 years old and had major depressive disorder. A multiplex immunoassay assessed 13 immunological biomarkers, and we examined their associations with structural MRI (grey matter volume and white matter hyperintensity volume (WMH)) and executive function (Color-Word Interference and Trail-Making tests) measures.

Results—Vascular endothelial growth factor (VEGF) and the chemokine eotaxin had significant negative associations with grey matter volume (VEGF: n=31, $r=-0.65$; eotaxin: n=29, $r=-0.44$). Tumor necrosis factor alpha (TNF- α) had a significant positive relationship with WMHs (n=30, $r=0.52$); Interferon- γ (IFN- γ) and macrophage inflammatory protein-1 α (MIP-1 α) were also significantly associated with WMHs (IFN- γ : n=31, $r=0.48$; MIP-1 α : n=29, $r=0.45$). Only eotaxin was associated with executive function (set-shifting performance as measured with the Trail-making test: n=33, $r=-0.43$).

Conclusions—Immunological markers are associated with brain structure in LLD. We found the immunological correlates of grey and white matter differ. Prospective studies are needed to evaluate whether these immunological correlates of brain health increase the risk of LLD's consequences. Eotaxin, which correlated with both grey matter volume and set-shifting performance, may be particularly relevant to neurodegeneration and cognition in LLD.

Keywords

depression; neuroimaging; MRI; immunology; inflammation; brain structure

There is a need to understand the specific pathophysiological processes which confer the consequences of late-life major depression (LLD), which include excess disability (Whiteford et al., 2013) and increased dementia risk (Diniz et al., 2013, Cherbuin et al., 2015). Since LLD is associated with pathology affecting both white and grey matter (Smagula and Aizenstein, 2015), brain structural pathology is likely to be a key driver of LLD's consequences. Indeed, cerebrovascular disease is well-recognized as a potential contributor to LLD's consequences (Alexopoulos et al., 1997); for example, evidence shows that LLD patients with a greater burden of cerebrovascular disease (measured by white matter hyperintensities volume (WMH)) are at increased risk for dementia (Steffens et al., 2007) and death (Levy et al., 2003). But there remains a need to understand the specific physiological processes which underlie brain structural pathology in LLD.

Altered immunological function has emerged as a potential contributor to the pathogenesis (Alexopoulos and Morimoto, 2011) and pathophysiology of LLD (Taylor et al., 2013), and specific immunological markers may have a role conferring LLD's consequences on brain health. Inflammatory cytokines are involved in the pathogenesis of atherosclerosis (Libby, 2006) and may contribute similarly to cerebrovascular pathology in LLD. Compared with healthy controls, patients with LLD have elevated levels of peripherally measured pro-inflammatory cytokines (e.g., tumor necrosis factor alpha (TNF- α) (Dowlati et al., 2010, Liu et al., 2012) and interleukin (IL-6) (Hiles et al., 2012, Dowlati et al., 2010, Liu et al., 2012)). Active lymphatic vessels were recently discovered in the brain (Louveau et al., 2015), and in aging, peripheral immune activation can trigger an exaggerated central nervous system response (Dilger and Johnson, 2008).

In the general population, pro-inflammatory cytokines in the periphery are associated cerebrovascular disease markers (Shoamanesh et al., 2015, Fornage et al., 2008). In LLD, one study found peripheral markers of inflammation were among the factors related to WMHs (Diniz et al., 2015), and another study found higher IL-6 levels were associated with lower grey matter volume in the hippocampus (Frodl et al., 2012). However, in addition to pro-inflammatory cytokines, a range of other immunological markers, including chemokines, may be altered in depression (e.g., see Simon et al. (2008), Grassi-Oliveira et al. (2012), Carvalho et al. (2015), Lehto et al. (2010), Magalhaes et al. (2014) and for reviews/meta-analyses see Stuart et al. (2015) and Eyre et al. (2016)). Given that a wide range of cytokines and chemokines associated with depression, there is a need for exploratory (hypothesis-generating) research to determine which of these immunological factors relate to biologically and clinically relevant aspects of brain health in LLD.

We therefore performed an exploratory pilot study to characterize the relationships between candidate immunological biomarkers with important aspects of brain health that are altered in LLD, namely, measures of brain structure (Du et al., 2014, Wang et al., 2014) and executive function (Dybedal et al., 2013). We used a multiplex assay to simultaneously measure levels of candidate immune markers that have been implicated in LLD pathophysiology (basic information regarding the known biological functions of this biomarker panel is provided in Supplemental Table 1). Our aim was to generate specific hypotheses regarding the potential immunological determinants of brain health outcomes in LLD. We hypothesized that specific immunological markers would be associated with both brain structure and executive function.

Methods

Participants

Participants were aged ≥ 60 and had current nonpsychotic major depressive disorder. Parent study recruitment has been described previously (Joel et al., 2013). Participants had major depressive disorder diagnosed by the Structured Clinical Interview for DSM-IV (First, 2002) plus a Montgomery Asberg Depression Rating Scale score ≥ 15 (Montgomery and Asberg, 1979). Exclusion criteria were: lifetime diagnosis of bipolar disorder, schizophrenia, schizoaffective, other psychotic disorders or current psychotic symptoms; clinical history of dementia or cognitive impairment as indicated by a score of ≥ 20 on the Mini Mental Status Exam (Folstein et al., 1975); alcohol or substance abuse in the past 3 months; high suicide risk; unstable medical illness; or contraindication to venlafaxine XR or aripiprazole. Eligible individuals were invited to participate in an associated MRI study, and the current report pertains to a subset of these participants with available MRI and blood biomarker data ($n=31$ total).

Measures

Morning blood samples were collected after an overnight fast at study baseline. Plasma biomarkers were initially assessed using a Meso Scale Discovery V-PLEX Human Cytokine 30-Plex Kit on a Quickplex-120 machine (Rockville, MD, USA) run accordingly to the manufacturer's protocol. The MSD multiplex platform allows for low sample volumes and provides a rapid, highly sensitive and reproducible system with a high dynamic range. Based on initial results, we identified 13 analytes with signal above background that were subsequently assessed (Supplemental Table 1). Acute health events (e.g. infection) may lead to temporarily elevated immunological biomarkers levels, therefore to avoid potentially misleading results in this small pilot study, we excluded blood biomarker values outliers defined as those exceeding the biomarker's 75th percentile value added to 3 times its interquartile range; only minimal data points met this criteria (2 interleukin-17A samples; 1 interleukin-2 sample; 2 interleukin-4 sample; 3 MCP-1 samples, 1 Eotaxin sample, 2 TNF- α samples; and 1 MIP-1 α sample).

MRI scanning was performed at the University of Pittsburgh, MR Research Center using a 3-T Siemens Tim Trio scanner with a Siemens 12-channel head coil. MPRAGE images were acquired on the axial plane: repetition time=2,300 milliseconds (ms); echo time=3.43 ms;

inversion time=900 ms; flip angle=9°; slice thickness=1 mm; field of view=256 × 224 mm²; voxel size=1×1×1 mm; matrix size=256 × 224; number of slices=176. Measures of whole brain gray matter were extracted using Automated Labeling Pathway (a method we developed to implement atlas-based segmentation of MR images (Aizenstein et al., 2005, Wu et al., 2006a)) and were normalized by dividing by total intracranial volume. FLAIR images were acquired on the axial plane: repetition time=9,160 milliseconds; echo time=89 milliseconds; inversion time=2,500 milliseconds; flip angle=150°; field of view=256 × 212 mm; slice thickness=3 mm; matrix size=256 × 240; number of slices=48; voxel size=1 × 1 mm. WMH volume was obtained from the MPRAGE and T2-weighted FLAIR images using an automated method for quantifying and localizing WMH (Wu et al., 2006b) using a fuzzy-connected algorithm (Udupa et al., 1997), and total brain white matter hyperintensities (WMH) was normalized by total brain volume. Of the subjects with completed assays (n=38), 6 did not participate in the MRI study, and one participant was excluded from the analysis because enlarged ventricles prevented their images from adequately warping into template space.

A neuropsychological test battery was administered and supervised by a senior neuropsychologist, MAB. We focused on executive function due to the known association with late-life depression (Dybedal et al., 2013), and previous research which suggests that executive function may exert “top-down” influence on other aspects of cognition (Morimoto et al., 2012). Two tests were used to evaluate executive function from the Delis-Kaplan Executive Function Scale (D-KEFS) (Homack et al., 2005)—the Color-Word Interference task (measuring response inhibition) and two Trail-Making tasks (measuring set shifting). Color-Word condition 3, called “inhibition,” assesses participant’s ability to inhibit an automatic response (i.e., reading words), where instead, participants must produce a response that requires more effort (i.e., naming the colors of words); we used a scaled score with permission from Pearson®. The Trail Making Test condition 4 (also known as the Number-Letter Switching condition) requires that examinees switch back and forth between connecting numbers and letters (i.e., 1, A, 2, B, etc., to 16, P). Condition 5 is a motor speed condition in which examinees trace over a dotted line connecting circles on the page as quickly as possible to gauge their motor drawing speed. In the present work, we compared performance on Condition 4 with performance on Condition 5; this removes the fine motor speed element from the test score, allowing ascertainment of cognitive flexibility independent of motor speed (Lezak et al., 2012).

Statistical analysis

We first assessed the distributions of the 13 blood and 4 brain health measures for normality, and applied log or square root transformations as needed. We assessed associations between baseline blood biomarkers with grey matter volume and executive function measures using Pearson correlations, and between blood biomarkers and WMH volume (which was not normally distributed even after transformation) using Spearman correlations. We present partial correlation coefficients as a measure of effect size adjusted for age, gender, and body mass index (which may be confounders of the association between immunological markers and brain health); associations with executive function were further adjusted for years of education. We also initially adjusted all models for the time of blood draw (in minutes from

midnight), which was not associated with blood biomarker levels, did not affect our estimates, and was dropped from subsequent models. Sensitivity analyses were conducted excluding influential data points identified using Cook's distance (Cook, 1977) (using linear regression diagnostic for associations of blood biomarkers and brain health measures). Spearman correlations were utilized when influential data-points were detected. We defined statistically significant effects as those with $p < 0.05$ and corrected for multiple comparisons in this exploratory pilot study using a Benjamin-Hochberg (Benjamini and Hochberg, 1995) false-discovery rate (FDR) threshold of $q < 0.30$ (as used previously for similar exploratory research (Diniz et al., 2015)).

Results

Basic demographic/clinical characteristics of MRI study participants with available blood biomarker data are shown in Table 1. Unadjusted correlation coefficients are available in Supplemental Table 2. Several small-to-moderately sized significant correlations were detected between blood biomarkers and measures of brain structure (significant age, sex, and BMI adjusted partial correlations are bold in Table 2). Vascular endothelial growth factor (VEGF) and eotaxin were both negatively correlated with grey matter volume. Tumor necrosis factor alpha (TNF- α), interferon-gamma (IFN- γ) and macrophage inflammatory protein 1-alpha (MIP-1 α) were positively correlated with WMH volume. Eotaxin was also negatively correlated with Trail-Making performance. None of the blood measures significantly correlated with performance on the Color-Word Interference task. All the associations reported (illustrated in Figure 1) were not substantively altered in sensitivity analyses excluding potentially influential outliers.

Discussion

We found multiple, distinct, immunological factors related to grey matter volume and WMH burden, and these findings highlight the complexity of the dialogue between peripheral immune function and brain health. These observations suggest separate, multifactorial immunological pathways contribute to neurodegeneration and white matter disease in LLD. In addition to these general observations, our results support several specific hypotheses regarding the pathophysiological determinants of poor brain health outcomes in LLD.

In our study eotaxin was associated with both worse set-shifting performance (an aspect of executive function measured with the Trail-Making test) and grey matter volume. Eotaxin levels are higher in patients with depression compared with controls (Grassi-Oliveira et al., 2012, Magalhaes et al., 2014, Simon et al., 2008), and experimentally increasing eotaxin levels in young mice decreases neurogenesis and impairs cognitive function (Villeda et al., 2011). This past literature importantly adds plausibility of the hypothesis, generated by our findings, that eotaxin may have a direct neurodegenerative effect related to executive function impairments in LLD.

We also found higher VEGF levels correlated with lower grey matter volume. VEGF increases the permeability of the blood brain barrier (Zhang et al., 2000) and might relate to grey matter volume by increasing the brain's exposure to neurotoxic factors. Alternatively,

VEGF is involved in neuro- and angio-genesis, therefore up-regulation of circulating VEGF could reflect an attempt to mitigate ongoing neurodegenerative processes (as opposed to a direct causal effect on grey matter volume). Of note, the correlation between VEGF and grey matter was similar in magnitude with a recent study of patients with schizophrenia ($r=-0.40$ in (Pillai et al., 2015), compared with our unadjusted finding of $r=-0.42$ (Supplemental Table 2)). Therefore, while the relationship between VEGF and grey matter volume is not mechanistically understood, these consistent findings suggest the relationship is not disease specific.

Higher TNF- α was related to greater WMH volume, consistent with *in vitro* evidence that TNF- α is related to myelin degeneration (Selmaj and Raine, 1988). We also observed that two other pro-inflammatory markers (interferon- γ and macrophage inflammatory protein-1 α) were correlated with greater WMH burden. These findings support hypotheses in the current literature that pro-inflammatory cytokines contribute to vascular disease affecting white matter in LLD (Taylor et al., 2013). Our findings add that the immunological underpinnings of brain health in LLD are likely to be multifactorial (i.e., potentially including multiple pro-inflammatory markers contributing to both demyelination and vascular disease). Targeting these pro-inflammatory pathways may be useful to prevent or attenuate the progression of white matter pathology, and may represent a mechanism by which TNF- α antagonism has an antidepressant effect in some patients (Raison et al., 2013) (i.e., by attenuating white matter disease progression).

It is important to recognize several limitations. Our analysis of associations between immunological and brain measures was cross-sectional in design, and this limits our ability to make causal inferences regarding the temporal sequencing of these relationships. To correct for multiple comparisons we applied a liberal FDR and findings with larger q-values have a greater chance of being false-positives. We detected several more significant results than expected by chance (with our false positive rate set at 0.05 and 13 blood biomarkers). Nevertheless, our study was designed to be hypothesis-generating and our findings must be replicated in confirmatory studies targeted to fewer comparisons in larger, independent samples. In addition, due to the small sample size our study has low statistical power and may have failed to detect true associations (type 2 error) that were of small effect or not apparent in our sample. Future research is needed to establish precisely whether/why only some immunological biomarkers, of many with somewhat similar functions (e.g., pro-inflammatory), are associated with brain health. Among cytokines/chemokines with related functions, it may be that a few (e.g., the identified markers) have functional distinctions that make them uniquely related to specific aspects of brain health. Alternatively, certain immunological pathways (e.g., multifactorial pro-inflammatory cascades) may be generally relevant to brain health, but of the measured markers in our study, only the individual associations of strongest magnitude were identified. Regarding generalizability, our sample consisted of mostly “young old,” white participants, and these findings will not necessarily generalize to other ethnic groups, younger adults, or the oldest old. Finally, our analysis did not identify the underlying source(s) of variability in these immunological biomarkers; although the associations identified were independent of BMI, our findings may not generalize to other groups wherein the putative drivers of these immunological cascades

(e.g., visceral fat leading to pro-inflammatory activation) are less prevalent (e.g., populations with less fat mass).

Conclusions

This exploratory pilot study identified specific immunological biomarkers (from a large pool of candidates previously linked to depression) that are associated with biologically/clinically relevant aspects of brain health in LLD. Taken with prior literature, our findings support several hypotheses that should be tested in future studies. First, eotaxin may increase the risk of both neurodegeneration and executive function impairment in LLD. Future studies should examine whether atrophy in specific regions mediates the association between eotaxin and set-shifting, and studies with prospective designs are needed to establish whether, in humans, eotaxin levels predict future grey matter changes. Second, VEGF may be related to neurodegeneration across diseases, but studies with repeated measures of both VEGF and brain structure are needed to evaluate the temporal sequencing of VEGF up-regulation and grey matter atrophy. Finally, our observations are consistent with the hypotheses that multi-factorial pro-inflammatory processes, notably marked by TNF- α , drive changes to white matter structure in LLD. Future studies are needed to clarify whether these pro-inflammatory markers exert a direct demyelinating effect, whether these inflammatory factors have effects through vascular pathology, and whether these potentially different pathways from physiology to white matter disease have clinical relevance. In conclusion, our study adds to evidence that multi-factorial immunological functions relate to poor brain health outcomes in LLD. Prospective and intervention studies are warranted to resolve whether modifying the identified immunological factors would reduce the consequences of LLD on the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key points

1. Multiple, distinct, immunological factors are related to grey matter volume and white matter hyperintensity burden in late-life depression (LLD)
2. The chemokine eotaxin related to both grey matter volume and executive function (set-shifting performance on the Trail-Making test), and may therefore be particularly relevant to neurodegeneration and cognition in LLD;
3. Multi-factorial pro-inflammatory processes, notably marked by TNF- α , are related to white matter disease in LLD;
4. Prospective and intervention studies are warranted to resolve whether modifying the identified immunological factors would reduce the consequences of LLD on the brain.

Figure 1. Scatter plots illustrating the significant associations between immunological markers and brain health detected

Top shows negative correlations between VEGF (n=31, $r=-0.42$, $p<0.0001$, $q<0.01$) and eotaxin (n=29, $r=-0.43$, $p=0.03$, $q=0.20$) with GMV (*as a proportion of total intracranial volume), as well as the negative correlation between eotaxin and Trail-Making test performance (TMT; n=33, $r=-0.39$, $p=0.02$, $q=0.24$); Bottom shows the positive correlations between IFN- γ (n=31, $r=0.36$, $p=0.02$, $q=0.15$), TNF- α (n=30, $r=0.62$, $p=0.01$, $q=0.07$), and MIP-1 α (n=29, $r=0.46$, $p=0.0477$, $q=0.21$) with WMH volume (*as a proportion of total brain volume). Negative correlation between eotaxin and Trail-Making test performance.

Table 1

Clinical and demographic characteristics of participants in the MRI study with available blood biomarker data (n=31)

Age	68.8 (5.5)
Female sex, % (n)	74 (23)
Caucasian race, % (n)	87 (27)
Body mass index	30.9 (6.7)
Color-Word Interference (condition 3) *	10.4 (2.6)
Trail-Making Test (condition 4 vs. 5)	8.8 (3.7)
Baseline MADRS	26.0 (6.2)

Mean (standard deviation) shown unless otherwise noted;

* Used with permission from Pearson[©]; MADRS, Montgomery–Åsberg Depression Rating Scale (Montgomery and Åsberg, 1979)

Table 2

Partial correlations between peripheral immunological biomarkers with brain structure and executive function measures

	Grey Matter Volume				White Matter Hyperintensities ³				Trail making test (condition 4 vs. 5 scaled score)				Color-Word Interference (condition 3)*			
	n	r	p	q	n	r	p	q	n	r	p	q	n	r	p	q
IFN- γ	31	-0.19	0.3461	0.7031	31	0.48	0.0096	0.0624	35	-0.05	0.8034	0.8585	35	-0.13	0.4980	0.8630
IL-2 ¹	31	-0.13	0.5054	0.8213	31	0.15	0.4388	0.7537	35	0.08	0.6868	0.8585	35	-0.08	0.6674	0.8630
IL-4	27	-0.09	0.6784	0.8422	27	0.05	0.8273	0.8742	31	-0.17	0.4094	0.7603	31	-0.29 ⁴	0.1420 ⁵	0.8376
IL-6 ¹	31	-0.04	0.8360	0.8422	31	0.12	0.5268	0.7537	35	0.24	0.2021	0.7603	35	0.14	0.4564	0.8630
IL-10 ¹	31	-0.25	0.1931	0.5021	31	0.19	0.3268	0.7081	35	0.07	0.7268	0.8585	35	0.04	0.8176	0.8630
TNF- α	30	-0.29	0.1474	0.4790	30	0.52	0.0055	0.0624	34	-0.16	0.4088	0.7603	35	-0.09	0.6182	0.8630
GM-CSF	31	-0.05	0.8005	0.8422	31	0.24	0.2095	0.6760	35	-0.03	0.8585	0.8585	35	-0.04	0.8409	0.8630
IL-12/IL-23p40 ²	31	-0.17	0.3786	0.7031	31	-0.03	0.8742	0.8742	35	-0.13	0.4777	0.7763	35	-0.11	0.5421	0.8630
IL-17a	30	-0.04	0.8422	0.8422	30	0.11	0.5798	0.7537	34	0.25	0.1876	0.7603	34	-0.24	0.1933	0.8376
VEGF	31	-0.65	0.0002	0.0026	31	0.22	0.2600	0.6760	35	-0.06	0.7673	0.8585	35	0.13	0.4909	0.8630
Eotaxin	29	-0.44	0.0259	0.1684	29	0.04	0.8508	0.8742	33	-0.43	0.0186	0.2418	34	0.30	0.8630	0.8630
MIP-1 α	29	-0.34	0.0844	0.3657	29	0.45	0.0209	0.0906	33	-0.19	0.3294	0.7603	34	-0.13	0.4935	0.8630
MCP-1	29	-0.12	0.5729	0.8275	29	-0.14	0.4869	0.7537	33	-0.23	0.2392	0.7603	34	0.27	0.1449	0.8376

Adjusted for age, sex, and body mass index (executive function associations also adjusted for education); All blood biomarkers were log transformed unless otherwise noted;

¹ Square root transformed;

² No transformation applied;

³ Spearman correlation used due to WMH distribution;

⁴ Spearman correlation used due to influential data-point;

⁵ P-value shown includes an influential data point defined using Cook's Distance (See Supplemental Methods), removing the single data point attenuates to p=0.2958; *Used with permission from Pearson©