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Short Communication

Gut permeability and cognitive decline: A pilot investigation in the Northern Manhattan Study

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A B S T R A C T

Background: Gut microbiota may impact cognitive function and decline, though data are limited. This pilot study examines the associations between gut dysbiosis products, plasma lipopolysaccharide (LPS) and soluble CD14 (sCD14), with cognitive decline and immune molecule activation among 40 participants in the longitudinal population-based Northern Manhattan Study.

Methods: We selected stroke- and dementia-free participants at baseline with high activation levels of core components of the immune signaling pathways underlying microbiota metabolite-cognitive associations (IL-1, IL-17, TNF). Participants were followed with up to three complete neuropsychological assessments, at least 5 years apart.

Results: Elevated sCD14 was associated with high levels of IL-1 pathway activation ($p < 0.05$), whereas in samples where only those molecules within the IL-17 and TNF pathways were increased, LPS and sCD14 levels were not elevated. LPS was associated with decline in global cognitive performance over 2–3 assessments (adjusted $\beta = -0.023$ per SD per year, 95% CI:-0.036, −0.010). The association between sCD14 and cognitive decline was marginal (adjusted $\beta = -0.018$ per SD per year, 95% CI:-0.040, 0.004).

Conclusions: These preliminary data support the hypothesis that gut dysbiosis leads to systemic and neuro-inflammation, and subsequently cognitive decline. Further large targeted and untargeted gut microbiota-derived metabolomic studies are needed.

The human gut is home to trillions of microbes that participate in a lifelong symbiotic relationship with their hosts and perform essential regulatory functions for health ranging from regulating nutrition and metabolism to the immune system (Clemente et al., 2012). Gut microbes impact health in part by metabolizing dietary and host-derived substrates, thereby generating biologically active compounds including signaling compounds, biological precursors, and toxins. (Tremaroli and Bäckhed, 2012). Recent studies implicate gut microbiota in the regulation of cognitive functions through a 'gut microbiota-brain axis' (Morais et al., 2020) that has a particular relevance in the elderly because it contributes to chronic low-grade inflammation and modulates age-related changes in innate immunity and cognitive functioning (O'Toole and Jeffery, 2015). Gut dysbiosis, disrupted intestinal integrity, and high serum levels of gut microbiota-derived metabolites have been linked to stroke and CVD (Wang et al., 2011) as well as to cognition, mild cognitive impairment and Alzheimer's Disease (MahmoudianDehkordi et al., 2019).

Gut dysbiosis disrupts gut barrier function and causes translocation to the circulation of bacterial products such as lipopolysaccharide (LPS), which leads to activation of monocytes and other myeloid cells, with release of proinflammatory markers including cytokines and soluble CD14 (sCD14) (Cavaillon et al., 2018). Our pilot study examines the relationship of LPS and sCD14 plasma levels with cognitive decline and levels of activation in immune molecules associated with three key pathways - interleukin-1 (IL-1), IL-17, and tumor necrosis factor (TNF) - that may be activated by gut dysbiosis products, govern mucosal barrier integrity, and have been implicated in the pathogenesis of cognitive deficits in some studies (Cipollini et al., 2019). We hypothesized that

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increased plasma levels of LPS and sCD14 – markers of gut dysbiosis and compromised intestinal integrity – would be associated with increased circulating pro-inflammatory immune molecules and with cognitive decline, specifically because cognitive decline might be driven by inflammation.

1. Methods

Participants were selected from the ongoing, prospective, community-based Northern Manhattan Study (NOMAS), designed to examine the incidence and risk factors for stroke, cognitive decline, mild cognitive impairment and dementia in a multi-ethnic, largely Hispanic urban population. From 1993 to 2001, participants were identified by random-digit dialing and recruited with the following eligibility criteria: a) never been diagnosed with a stroke; b) 40 years old or older; and c) resided in Northern Manhattan for ≥3 months, in a household with a telephone. Out of 3298 participants, 37% were men, 24% black, 21% white, 52% Hispanic, and the mean age at recruitment was 69 ± 10 years. From this cohort we selected 40 healthy stroke- and dementia-free participants with high levels of activation of the IL-1, IL-17 and TNF signaling pathways, three of the immune pathways most commonly proposed as either mediators or modulators of microbiota metabolite-cognitive associations and as regulators of intestinal barrier permeability (Cipollini et al., 2019). Molecules from these 3 pathways were selected from a 60-plex immune marker panel (see full list below of all IL-1, IL-17 and TNF pathway-associated molecules). Data distributions and patterns of overlap across all pathway-relevant molecules were examined to derive cutoffs. To address the centrality of IL-1β, IL-17A, and TNF-α in terms of their known effects in the intestinal tract and in regulating the integrity of the epithelial barrier, their interactions with immune cells that influence microbiota, and their high degree of correlation with the other immune molecules in the same pathway (Cipollini et al., 2019), we defined activation of the 'core' representative molecules for each of the 3 pathways as levels >75th percentile. We then created seven non-overlapping groups (abscissa of the graph), with each group defined by activation of both the pathway-specific core molecule (IL-1β, IL-17A, or TNF-α) and activation of one or more molecules from the 3 pathways with levels above 2 standard deviation (SD) above the group mean; the 8th group comprised non-overlapping reference subjects without evidence of activation of these pathways (control). The final study sample of 40 subjects was formed by a random selection of 5 individuals meeting criteria for each of the eight groups.

NOMAS has been described in prior publications, including details on neurocognitive battery (Wright et al., 2015). We used the composite cognitive score created from four cognitive domains (executive function, memory, language, and processing speed). The first complete neuropsychological assessment was conducted starting in 2003, on average 7 ± 2 years after the NOMAS cohort was recruited, and the second complete neuropsychological assessment was conducted on average 5.2 ± 0.4 years after the first assessment, and the third complete assessment was conducted on average 12 ± 1 years after the first assessment. Thirty five out of the 40 participants in the current study had two complete neuropsychological assessments; of these 35, 19 had each completed three assessments.

Plasma LPS and sCD14 levels were analyzed using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis) as previously described (Zhang et al., 2019). Immune molecules (IL-1 pathway: *core pathway representative*, IL-1β; *other pathway participants*: IL-1α, IL-1 receptor antagonist, IL-6, IL-18, IL-31, leukemia inhibitory factor [LIF]; IL-17 pathway: *core pathway representative*, IL-17A; *other pathway participants*: IL-17F, IL-22, IL-21, IFN-γ, TGF-β; TNF pathway: *core pathway representative*, TNF-α; *other pathway participants*: TNF-β [lymphotoxin-α], sFas ligand [sFasL], TRAIL [TNFSF10], CXCL8 [IL-8], colony stimulating factor 2 [CSF2, granulocyte-macrophage colony-stimulating factor]) were measured using a magnetic, bead-based 60-plex immunoassay (Affymetrix/eBioscience, Santa Clara) (Konishi et al., 2020).

We calculated means ± SD of LPS and sCD14 in plasma samples in the activated immune molecule groups defined above. The association between LPS and sCD14 was assessed by linear regression. Parametric statistical differences between the two immune molecule groups were established with a t-test, and non-parametric differences between these groups were established with a Mann-Whitney U test. Generalized estimating equations (GEE) were used to estimate the association of LPS and sCD14 with longitudinal cognitive scores adjusting for age, sex, race/ethnicity, and education. Interaction terms between visit time and LPS and sCD14 was introduced in the GEE model, and associations between baseline cognitive scores and the plasma levels were evaluated as main effects in the model.

2. Results

In our pilot sample (N = 40, mean age = 65 ± 9 years, 68% women, 68% Hispanic, 13% non-Hispanic Black, 20% non-Hispanic White), LPS ranged from 79-224 (mean = 176+/-33) pg/ml and sCD14 from 1200-2400 (mean = 1597+/-310) ng/ml. For every pg/ml increase in LPS, sCD14 increased by 2.40 ng/ml (SE = 1.48, p = 0.11).

We observed elevated levels of sCD14 ($p < 0.05$) only in groups with high IL-1 levels (above 2SD; Fig. 1A). Conversely, in samples where only IL-17 and TNF were increased, and not IL-1, LPS and sCD14 levels were not significantly elevated (Fig. 1B).

LPS and sCD14 were not significantly associated with the composite cognitive score at baseline (LPS: β = 0.082/SD, 95% CI:-0.14, 0.31; sCD14: β = -0.031/SD, 95% CI:-0.21, 0.15).

Increased levels of LPS and sCD14 were significantly associated with cognitive decline. In adjusted models, LPS was directly associated with cognitive decline (adjusted β = -0.023 per SD/year, 95% CI:-0.036, −0.010). The association between sCD14 and cognitive decline was marginal (adjusted β = -0.018 per SD/year, 95% CI:-0.040, 0.004).

3. Discussion

This pilot study provides evidence for the association between two 'leaky gut' markers, LPS and sCD14, and cognitive decline. Our observed elevated levels of sCD14 among individuals with high levels of IL-1 indicates a role of sCD14 in the IL-1 inflammatory pathway. This preliminary evidence suggests that gut microbiota-derived markers of gut permeability may drive systemic inflammation to levels that affect neuroinflammation and subsequent cognitive dysfunction.

Gut microbial dysbiosis and gut permeability ('leaky gut') increase the bioavailability of circulating microbial products that affect organs via activation of inflammatory signaling pathways. Lipopolysaccharide is an endotoxin produced by gram negative bacteria and a major driver of inflammation through activation of systemic monocytes and microglial cells, contributing to sustained neuroinflammation and neurodegeneration (Zheng et al., 2020). High levels of circulatory sCD14 as a marker of monocyte activation are reflective of LPS exposure, although not strictly restricted to LPS activation (Shive et al., 2015). In the Framingham Heart and the Cardiovascular Health Study, higher levels of sCD14 were associated with a higher risk of dementia and brain aging MRI markers, and with decline in executive function (Pase et al., 2020). The highest sCD14 levels were associated with risk of dementia independent of vascular risk factors and circulating inflammatory markers such as CRP or IL-6, suggesting sCD14 as an important inflammatory marker of dementia.

IL-1 is an inflammatory cytokine with diverse physiological functions that is important in innate immune processes (Dinarello, 2009). Small, localized elevations in IL-1 may be sufficient to drive potent neuroinflammatory changes in the brain, affecting memory and other cognitive processes (Rachal Pugh et al., 2001). The centrality of IL-1 elevations with higher sCD14 in plasma likely reflects shared upstream activators, such as LPS, that are involved in complex regulation through actions at toll-like receptors and at the NLRP3 inflammasome (Rachal Pugh et al.,

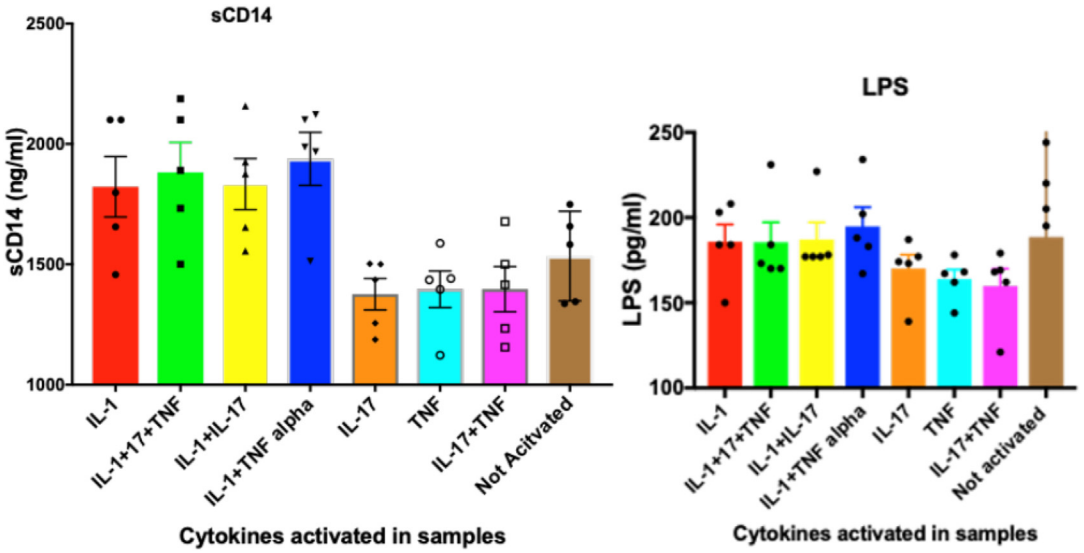

Fig. 1. Plasma levels of LPS and sCD14 in cytokine activated samples.

2001). Emerging understanding of IL-1's function has identified possible roles in triggering innate and adaptive immune responses and their relative contributions to beneficial vs. detrimental outcomes in chronic vascular and neurodegenerative diseases. Our study suggests a possible link between IL-1 and gut dysbiosis with a potential paradigm shift in the understanding of balance between the beneficial and deleterious IL-1 functions.

A limitation is the small number of selected markers and participants. However, we purposely targeted our markers to gain preliminary data on the potential link between gut microbiota-derived markers and systemic inflammation that may lead to neuroinflammation and cognitive dysfunction. Since clinical studies on these markers are sparse, our preliminary results are valuable and promising. Larger targeted and untargeted gut microbiota-derived metabolomic studies are needed to better understand the 'gut microbiota-brain axis', its role in neurovascular and neurocognitive dysfunction, and to identify related targets for disease-modifying interventions.

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Disclosures

None.

Declaration of competing interest

We have no conflicts of interest to disclose.

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