Interleukin 9 alterations linked to Alzheimer disease in African Americans

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Abstract

OBJECTIVE:

Compared to older Caucasians, older African Americans have higher risks of developing Alzheimer disease (AD) and lower cerebrospinal fluid (CSF) tau biomarker levels. It is not known whether tau-related differences begin earlier in life or whether race modifies other AD-related biomarkers such as inflammatory proteins.

METHODS:

We performed multiplex cytokine analysis in a healthy middle-aged cohort with family history of AD (n = 68) and an older cohort (n = 125) with normal cognition (NC), mild cognitive impairment, or AD dementia. After determining baseline interleukin (IL)-9 level and AD-associated IL-9 change to differ according to race, we performed immunohistochemical analysis for proteins mechanistically linked to IL-9 in brains of African Americans and Caucasians (n = 38), and analyzed postmortem IL-9-related gene expression profiles in the publicly available Mount Sinai cohort (26 African Americans and 180 Caucasians).

RESULTS:

Compared to Caucasians with NC, African Americans with NC had lower CSF tau, p-Tau181 , and IL-9 levels in both living cohorts. Conversely, AD was only correlated with increased CSF IL-9 levels in African Americans but not Caucasians. Immunohistochemical analysis revealed perivascular, neuronal, and glial cells immunoreactive to IL-9, and quantitative analysis in independent US cohorts showed AD to correlate with molecular changes (upstream differentiation marker and downstream effector cell marker) of IL-9 upregulation only in African Americans but not Caucasians.

INTERPRETATION:

Baseline and AD-associated IL-9 differences between African Americans and Caucasians point to distinct molecular phenotypes for AD according to ancestry. Genetic and nongenetic factors need to be considered in future AD research involving unique populations.

Older African Americans are more than twice as likely to develop Alzheimer disease (AD) dementia as older Caucasians. There are multiple potential causes for this, including the variable cognitive correlates for genetic AD risk factors, comorbid cerebrovascular and systemic diseases, socioeconomic status, and psychosocial inequity. Clinicopathologic studies of AD in African Americans are rare due to low consent rates for autopsy,[1] but available studies suggest greater prevalence of nonplaque/tangle copathology in African Americans.[2, 3] We and others have used antemortem biomarkers (cerebrospinal fluid [CSF], magnetic resonance imaging) to interrogate candidate mechanisms that may account for risk and phenotypic differences according to race. Whereas some AD biomarkers (eg, CSF β-amyloid 1-42 [Aβ42]) exhibited the same alteration patterns in African Americans and Caucasians, other related biomarkers showed opposite directions of change (eg, frontoparietal functional connectivity) or attenuated changes in the same direction (eg, CSF tau-related proteins).[4] We thus hypothesize that, in addition to differential risks, older African Americans and Caucasians develop divergent downstream (pathologic and neuroprotective) processes upon cerebral amyloid deposition. However, these findings do not rule out aging-related differences, and here we sought to confirm previously identified CSF biomarker differences in a middle-aged group of cognitively normal African Americans and Caucasians.

We also sought to identify inflammation-related biomarkers whose levels differ between the two races in middle and old age. Inflammation has been implicated in AD through genome-wide association,[5-7] natural history,[8-10] and neuropathologic studies.[11-13] Race likely modifies baseline immune surveillance and inflammatory responses, as North[14] and South[15, 16] Americans of African descent have more severe neuroinflammatory disorders (including multiple sclerosis, neuromyelitis optica, and idiopathic inflammatory disorders) than Caucasians with the same diagnoses. We previously found lower CSF levels of soluble vascular cell adhesion molecule 1 in African Americans than Caucasians across the cognitive spectrum, which was counterintuitive given the epidemiologic observation of more severe neuroinflammation. This can potentially be explained by elevated levels of other CSF cytokines. Alternatively, the relative change in CSF inflammatory biomarkers may better predict disease severity than their absolute levels. Although increased CSF levels of chemokines and cytokines have been previously reported in AD,[17, 18] the effect of race on the difference between normal cognition (NC) and AD has not been rigorously evaluated. Therefore, in addition to validating racial differences in CSF tau biomarker levels, we also examined race-dependent and race-independent CSF cytokine alterations. We then performed follow-up neuropathologic analyses in 2 autopsy cohorts, with a focus on interleukin (IL)-9 because its AD-related alteration was only seen in African Americans, and its expression is regulated by the transcriptional factor PU.1 encoded by the AD risk gene SPI1.[19]

Subjects and Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The protocols were approved by the Emory University Institutional Review Boards and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 1964 Declaration of Helsinki. Middle-aged African American and Caucasian subjects

were recruited into a study of cognitively normal subjects with a family history of AD dementia (principal investigator: W.W), and older African American and Caucasian subjects were recruited into a study of NC, mild cognitive impairment (MCI), and AD dementia as previously described (principal investigator: W.T.H.).4 Written informed consents were obtained from all participants as well as their legal representatives when appropriate. The studies were registered at ClinicalTrials.gov under NCT 02471833 (W.W.) and NCT 02089555 (W.T.H.).

Subjects and Preanalytical Processing

Demographic (age, sex, education), diagnostic (syndrome, global Clinical Dementia Rating, Mini-Mental State Examination), and APOE allelic information were collected (Table 1). CSF (20ml) was collected using protocols modified from the Alzheimer's Disease Neuroimaging Initiative20 using 24G Sprotte atraumatic needles and syringes between 8 am and noon without overnight fasting, and transferred into two 15ml polypropylene tubes. For the middle-aged cohort, CSF was centrifuged at 2,000 rpm for cellular studies; the supernatant was removed, immediately aliquoted (500µl), labeled, and frozen (−80°C) until analysis. For the older cohort, CSF was not centrifuged, and was immediately aliquoted (500µl), labeled, and frozen (−80°C) until analysis. We previously prospectively determined that 2,000 rpm centrifugation did not influence cytokine levels measured here.21

Table 1. Demographic Features of Subjects Included in the Current Study

Subject Grouping

Each subject was categorized according to clinical diagnosis (NC, MCI, AD dementia; see Table 1). Established CSF AD biomarkers (Aβ42, total tau [t-Tau], and tau phosphorylated at threonine 181 [p-Tau181]) were measured in the middle-aged cohort (n = 68) using enzyme-linked immunosorbent assays (Fujirebio, Ghent, Belgium) in the Zetterberg laboratory, and the same markers were previously measured in the older cohort using Luminex-based multiplex assays (Fujirebio US, Malvern, PA) in Atlanta. We have performed cross-platform validation studies at Emory to show strong linear correlation between these two platforms.22

CSF Cytokine and Chemokine Assays

Seven inflammatory proteins were selected for their preferential association with innate immunity or different immune cell populations (including T-helper [Th] cells 1, 2, 9, and 17), including tumor necrosis factor (TNF)-α, macrophage-derived chemokine (MDC/CCL22), IL-7 (microglia), IL-8 (IL-8/CXCL8, Th17), IL-9 (Th9), and IL-10 (Th2). Interferon gamma (Th1) levels were not consistently detectable. In its place, a downstream marker interferon gamma-induced protein 10 (IP-10/CXCL10) was used as a surrogate. All these proteins were measured in a Luminex 200 platform using the Merck-Milliplex MAP Human Cytokine Panel (HCYTOMAG-60K; Merck-Millipore, Burlington, MA) following the manufacturer's protocol except that two 100µl aliquots of CSF were used for duplicates. All operators were blinded to the diagnosis, and final assay results were then analyzed according to race, age, and diagnosis. In our laboratory, we achieve average intermediate precision (over experiments performed over 9 days) of 9.4% for TNF-α, 12.9% for MDC, 14.7% for IL-7, 4.8% for IP-10, 12.0% for IL-10, 9.2% for IL-9, and 7.6% for IL-8. Freeze-thawing experiments23 using CSF from

6 separate subjects showed significant degradation over 2 freeze–thaw cycles for MDC and TNF-α (p = 0.021 and p = 0.012 for slope in exponential decay), and we previously showed light centrifugation21 to have minimal impact on these CSF cytokine levels.

Immunohistochemistry

To extend CSF-based differences in IL-9 to the brain, we performed immunohistochemistry (IHC) analysis on brains from 38 subjects (19 African Americans and 19 Caucasians; Table 2) from the Emory Alzheimer's Disease Research Center Brain Bank. African American cases were selected based on availability and exclusion of major non-AD pathology (eg, intraparenchymal hemorrhage); Caucasian cases were age- and gender-matched to the African American cases. Both IHC and subsequent quantitative analysis were performed with the operators blinded to each case's race and diagnosis. Formalin-fixed superior middle frontal cortical sections were stained with the Lab-Vision Auto Stainer 480S platform (Thermo Fisher Scientific, Waltham, MA) using primary antibodies targeting IL-9 (66144-1, 1:1,500; EMD Millipore, Billerica, MA), CD3 (a marker for T-cells, ab5690, 1:500; Abcam, Cambridge, MA), PU.1 (a known transcription for Th9 cells, E.388.3, 1:500, Thermo Fisher Scientific), and tryptase (a mast cell marker related to Th9, M444905, 1:2,000, EMD Millipore) followed by diaminobenzidine (DAB)-based detection. Slides were digitally scanned using the Aperio Digital Pathology Slide Scanner (Leica Biosystems, Buffalo Grove, IL). Quantitative pathologic analysis was performed using Aperio Image Scope (12.3.3.5048) to generate total area of positive staining. For each slide, a 100 × 80 grid was created for the coverslip to create 8,000 fields of 50μM × 50μM, and 25 fields were selected through randomly generated coordinates. A randomly selected field was analyzed using Aperio ImageScope if (1) more than half of the field was occupied by cortex and/or white matter, (2) there was no large mounting artifact (tear, fold), and (3) there was no significant staining artifact (eg, DAB debris). Each case's mean immunoreactive area across 25 fields was then used for group-level statistical analysis.

Table 2. Demographic Information for the Emory Neuropathology Cohort

Statistical Analysis

Statistical analysis was performed by SPSS 24 (IBM, Armonk, NY) with effect sizes, 95% confidence intervals (CIs), and p values related to race, AD, or their interaction (if significant) shown in Table 3. Model fit for the relationship between IL-9 and t-Tau (or p-Tau181) was analyzed using Prism 8.0 (GraphPad, San Diego, CA). For baseline comparison between the two races in the middle-aged group (all NC), chi-squared tests (for categorical variables) and Student t tests (for continuous variables) were used with $p < 0.01$ to adjust for multiple comparisons. For comparison between biomarker levels in this group, analysis of covariance (ANCOVA) was first used to detect differences in established AD biomarkers (Aβ42, t-Tau, p-Tau181) and cytokines between races. All linear regression and ANCOVA were adjusted for age (age at death for the autopsy cohorts) and sex. Because some CSF cytokines correlate with tau biomarker levels, we also analyzed the relationship between cytokines and t-Tau (or p-Tau181) first through linear analysis, followed by the identification of possible inflection points through segmental linear regression (Prism 8.0) and iteratively selected inflection points.

Table 3. Summary of Relationships between Race, CSF Cytokines, and AD

CSF IL-9 levels were then analyzed in the older cohort of subjects with NC, MCI, and AD in relationship to race, AD diagnosis, cognition, and other Th-related cytokines. Student t test was first used to determine whether CSF IL-9 levels differed between older African Americans and Caucasians with NC, and then between NC and AD subjects of the same race. Linear regression analysis was used to determine whether race modified IL-9 levels, the relationship between cognition and IL-9, or both. The same was completed for other cytokines (IP-10, IL-4, IL-10). The difference between race-normalized (through z-transformation) IL-9 and IP-10 levels was finally calculated for each subject to derive a measure for Th1-to-Th9 bias, and linear regression was used to examine whether race modified the relationship between cognition and this bias.

For IHC analysis, ANCOVA was used to analyze areas of IL-9 and PU.1 immunoreactivity according to race and diagnosis (NC vs AD). Mean brain IL-9–immunoreactive areas (pixels; averaged over 25 brain regions) were analyzed directly, and mean brain PU.1- and tryptase-immunoreactive areas were log10-transformed because of non-normal distribution. An interaction term of race × diagnosis was introduced to determine whether AD-associated IL-9, log10(PU.1), and log10(tryptase) areas differed between the two racial groups.

For comparison with an independent cohort, the publicly available proteomic and gene expression dataset from the Mount Sinai cohort (New York, NY) was analyzed because of its inclusion of African Americans (n = 26) and Caucasians (n = 180) with NC, MCI, and AD.24 Because detailed plaque density was available, we performed linear regression analysis to examine whether race modified the relationship between neuritic plaque density and IL-9–related genes (including SPI1 for PU.1, IL4RA, IL33, TGFBR2, STAT6, SMAD3, and OX40 genes associated with Th9 differentiation; and KIT as a mast cell receptor; IL9 and TPSAB1 [for mast cell tryptase beta] mRNA were not detected). As TGFBR2 and IL4RA expression levels were already log-transformed, the ratio of TGFBR2 to IL4RA was calculated by deriving the difference between log-transformed values.

Results

Race Modified the Relationship between CSF IL-9 and Tau Biomarkers

We previously reported in the older cohort that African Americans had lower CSF t-Tau and p-Tau181 levels than Caucasians within each diagnostic category. In a separate younger cohort (all with NC), analysis using a singleplex enzyme-linked immunosorbent assay in an independent laboratory also showed lower CSF t-Tau (by 126pg/ml, 95% CI = 51–201, p = 0.001) and p-Tau181 (by 15.7pg/ml, 95%

CI = 6.4–25.0, p = 0.003) levels in African Americans than Caucasians, adjusting for age and sex, despite similar Aβ42 levels (by 17pg/ml, 95% CI = −87 to 120, p = 0.750; Fig 1).

Cerebrospinal fluid (CSF) Alzheimer disease biomarker and cytokine levels in middle-aged African Americans and Caucasians with normal cognition. Compared to Caucasians (open circles), African Americans (filled circles) had lower total tau (t-Tau; A*) and tau phosphorylated at threonine 181 (p-Tau181; B*) levels, but similar β-amyloid 1-42 (Aβ42; C) levels. The relationship between CSF interleukin (IL)-9 and t-Tau (D) or p-Tau181 (E) also differed according to race (top panels, blue lines for African Americans, green lines for Caucasians), and segmental linear analysis showed similar relationship between races at low t-Tau and p-Tau181 concentrations but possible divergence at higher concentrations. Shaded areas represent 95% confidence interval of the regression curves.

In this middle-aged cohort, African Americans had lower CSF IL-7 (by 0.48pg/ml, 95% CI = 0.08–0.88, p = 0.021) and IL-9 (by 1.11pg/ml, 95% CI = 0–2.22, p = 0.050) levels than Caucasians. However, these findings may be mediated by a strong correlation between CSF p-Tau181 and soluble cytokine levels, which we have previously observed in neuroinflammatory conditions such as multiple sclerosis and human immunodeficiency virus (Ozturk and Hu, unpublished data). Thus, we next analyzed the relationship between race, tau markers, and IL-9. We found IL-9 to demonstrate nonlinear relationships with both t-Tau and p-Tau181 in Caucasians (see Fig 1D, E, top panels). Segmental linear analysis showed inflection points only for Caucasians (see Fig 1D, E, lower panels), with similar relationships between the two races at low t-Tau and p-Tau181 concentrations but possible divergence at higher concentrations.

AD Dementia Associated with Higher CSF IL-9 Levels in African Americans

Although CSF IL-9 levels appeared to plateau with greater tau markers in Caucasians with NC, there remains the possibility that IL-9 levels in African Americans may also plateau beyond a higher t-Tau or p-Tau181 threshold. We thus next analyzed the effect of AD, which is associated with higher t-Tau and p-Tau181 levels, on Th-9–related CSF proteins in a previously recruited older cohort of African Americans and Caucasians4 with NC, MCI, and AD. Older African Americans with NC also had lower CSF IL-9 levels than Caucasians with NC (by 1.90pg/ml, 95% CI = 0.87–2.94, p = 0.001), and AD diagnosis was only associated with greater CSF IL-9 levels in African Americans (by 1.27pg/ml, 95% CI = 0.35–2.19, p = 0.010). Analysis using a continuous measure of cognitive function (Fig 2B) controlling for age and sex also showed greater IL-9 in AD only in African Americans (increase of 0.552pg/ml for each standard deviation of cognitive impairment, 95% CI = −0.076 to 1.179, p = 0.066).

Cerebrospinal fluid (CSF) cytokine levels in older African Americans and Caucasians according to diagnosis (A, C, E) or a continuous measure of cognition (B, D, F). CSF interleukin (IL)-9 levels were lower in cognitively normal African Americans (closed circles) than Caucasians (open circles), and were associated with increases in mild cognitive impairment (MCI) and Alzheimer disease (AD) dementia only in African Americans (A, B). Analysis of the balance between IL-9 and interferon gamma-induced protein (IP)-10 showed similar AD-associated Th1-to-Th9 bias, with Caucasians showing a greater overall bias than African Americans. NC = normal cognition. *See Table 3 for effects of race and cognitive z-score on biomarker levels.

To better characterize cytokine alterations according to race and AD, we additionally analyzed CSF levels of IP-10, IL-4, and IL-10 associated with Th1, Th2, and Th2/Th9 pathways. AD dementia was associated with lower Th1-related IP-10 levels only in Caucasians (see Fig 2C), but race did not significantly modify the effect of cognition when the latter was analyzed continuously (see Fig 2D; decrease of 0.417pg/ml for IP-10 for each standard deviation of cognitive impairment, 95% CI = 0.015–0.828, p = 0.035). On the other hand, Th2-related IL-4 levels were too low for detection in this cohort, and Th2/Th9-related IL-10 levels did not differ according to race or cognition in the older cohort (consistent with previously reported U-shaped curve for IL-10).[21] Thus, while AD was associated with increased Th9 activity only in African Americans, it may be associated with decreased Th1 activity only in Caucasians.

To further test this hypothesis, it is useful to examine the relative differences between cytokines belonging to separate pathways through normalization, which we have found to control for proinflammatory changes associated with aging (ie, inflammaging) within each individual.[25] To do this, we first transformed IL-9 and IP-10 into z scores according to a large cohort of cognitively normal adults recruited and analyzed at our university. We then derived a measure of IL-9 relative to IP-10 by subtracting z-transformed IL-9 level by z-transformed IP-10 level. This normalized measure then accounts for each cytokine's concentration ranges as well as factors that alter the two cytokines equally. This analysis revealed that, although race modified the relationship between each cytokine and cognition, African Americans persistently had less Th1-to-Th9 bias than Caucasians, although this bias increased with progressive cognitive impairment (see Fig 2E, F; slope = 0.212, 95% CI = 0.019–0.406, p = 0.018).

Race Modified Downstream, but Not Upstream, Marker of IL-9 Function

Based on our findings so far, we hypothesized that African Americans had lower baseline brain IL-9 levels than Caucasians in the absence of AD pathology, but similar brain IL-9 levels to Caucasians in the setting of AD. To test this, we performed IHC analysis in an independent autopsy series of African Americans and Caucasians with NC or AD and identified two patterns of IL-9 staining in the brain (see Table 2). Many cells on the abluminal side of small vessels were immunoreactive to IL-9 as well as the T-cell marker CD3, suggesting these to be perivascular Th9 cells (Fig 3A–C). Less intense IL-9 staining was identified in glia as well as neurons (see Fig 3D), and these were not immunoreactive to CD3. Quantitative analysis of scanned slides taking into account both perivascular and parenchymal immunoreactivity showed African Americans with NC to have lower mean and median brain IL-9 levels than the other groups, but the large brain IL-9 variance required a much larger cohort to demonstrate statistical significance (Fig 4A).

Immunohistochemistry of protein markers related to Th9 in postmortem brain tissue. (A–C) In Alzheimer disease (AD), there was intense staining of interleukin (IL)-9 in perivascular cells (A), which are also immunoreactive to T-cell marker CD3 (B) in adjacent slides, suggesting these to be IL-9–containing T cells (higher magnification in C). (D) There was also modest immunoreactivity to IL-9 in nonperivascular parenchymal neurons and glia, associated with increased PU.1 immunoreactivity (same magnification as IL-9) and strongly tryptase-positive cells (arrow). NC = normal cognition.

Mean superior frontal interleukin (IL)-9 (A), PU.1 (B), and mast cell tryptase (C) immunoreactive areas in a postmortem cohort from Emory. For each marker, immunoreactivity was normalized to the mean of Caucasian normal cognition (NC) subjects. Greater PU.1 was associated with Alzheimer

disease (AD; asterisk), whereas greater tryptase was only associated with African Americans with AD (dagger). Bars represent median values.

IL-9 expression in neurons in a disease associated with neuronal loss may in part account for the wide variance we observed. We thus additionally examined up- and downstream markers of IL-9 functions. PU.1 is a critical transcription factor for Th9 differentiation, and we found AD to be associated with elevated brain PU.1 regardless of race (see Figs 3D, 4B). In contrast, when we examined mast cells, which represent major effectors of IL-9 in other organs,[26, 27] we found AD to be associated with higher brain tryptase levels only for African Americans but not Caucasians (see Figs 3D, 4C; p = 0.063 for race × diagnosis interaction).

Brain IL4RA and KIT Profiles in African Americans Support Th9 Polarization in AD

Finally, we sought to replicate our findings in a separate clinicopathologic cohort of NC, MCI, and AD cases.[24] Proteomic analysis in the Mount Sinai cohort (26 African Americans, 180 Caucasians) did not detect IL-9 or any of the proteins in its network, consistent with our and others' experience that only abundant proteins are reproducibly detected using an untargeted approach.[28] Brain transcriptomic analysis on 7 genes previously implicated in the IL-9 network showed SPI1 (encoding PU.1, p = 0.030) to positively correlate with plaque burden independent of race, consistent with the brain IHC results from Emory for PU.1. When we examined 2 receptors (IL4RA, TGFBR2) linked to the differentiation of Th2 into Th9 cells, we found race to modify the relationship between plaque burden and IL4RA but not TGFBR2 (which increased with plaque density independent of race, p = 0.007). Compared to Caucasians, African Americans had greater baseline IL4RA expression (Fig 5A; $F_{1, 202}$ = 5.480, p = 0.020), which decreased with greater neuritic plaque burden only in African Americans ($F_{1, 202}$ = 5.441 for race × plaque burden, p = 0.021).

Relationship between neuritic plaque density, race, and interleukin 9–related transcripts in the Mount Sinai cohort. (A) For 2 genes involved in Th9 differentiation, race modified the relationship between plaque density and IL4RA but not TGFBR2 (Caucasians: open circles, dashed line; African Americans: filled circles, solid line). (B) Analyzing the ratio of TGFBR2 to IL4RA (TGFBR2/IL4RA) as a marker of Th9 differentiation showed a strong positive correlation with plaque density in African Americans but not Caucasians. (C) Examination of mast cell (effector of Th9 cells) surface marker KIT/CD117 revealed the same changes as TGFBR2/IL4RA ratio.

Because an optimal combination (not necessarily equal concentration) of transforming growth factor (TGF)-β and IL-4 exposure is needed for in vitro Th9 differentiation but the in vivo ratio is not known, we hypothesized that AD-associated Th9 differentiation was accompanied by an altered TGFBR2-to-IL4RA ratio. Whereas this ratio was independent of plaque density in Caucasians, it increased linearly with greater plaque density in African Americans (p = 0.009; see Fig 5B). A nearly identical trend was observed when we examined expression of the mast cell receptor KIT (see Fig 5C). Taken together, we interpret these findings to associate a higher TGFBR2-to-IL4RA ratio with Th9 differentiation, which in turn enhances the activation of mast cells as a main effector of Th9 signaling.

Discussion

The molecular basis for different clinical AD phenotypes between older African Americans and Caucasians is poorly understood. We previously identified lower CSF tau biomarker levels in older African Americans than older Caucasians regardless of diagnosis, and an opposite pattern of functional connectivity change involving brain areas perhaps more associated with tau than amyloid deposition.4 Here, we extended our findings to a middle-aged healthy cohort, and identified a new correlation between CSF IL-9 and AD-related changes only in African Americans. Furthermore, immunohistochemical and transcriptomic analysis of brain sections from 2 independent US cohorts revealed findings associated with Th9 differentiation and mast cell activation primarily in African Americans with AD. Taken together, we interpret these findings to support an AD-associated molecular phenotype switch between Th2 and Th9 in African Americans but not Caucasians.

IL-9 was previously thought to be released by Th2 cells, but is now recognized to originate from differentiated Th9 cells as well as Th17 cells and microvascular pericytes.29-31 The pleiotropic effects of IL-9 have been implicated in neurological (eg, multiple sclerosis32, 33 and atopic myelitis34) as well as systemic (eg, psoriasis35 and colitis36) disorders. IL-9 and its receptor have been found in neurons of the developing brain.37 To the best of our knowledge, this is the first report of neurons expressing IL-9 in mature brains. IL-9 is known to stimulate primary astrocytes and oligodendrocyte precursors without influencing microglial cells,38 but neurons can also themselves regulate T cells to release IL-9.39 Thus, IL-9 may represent a critical link in the cross-talk between neurons and perivascular T cells in the brain. Furthermore, IL-9 pathway was identified as a key point of genetic divergence between sub-Saharan Africans and African Americans,40 but it was not previously known how IL-9 levels varied between people of European versus African ancestry. Changes related to IL-9 have not been consistently reported in AD,41, 42 but there is intriguing animal data that knockin APOE ε4 allele in mice elicits greater IL-9 production than the wild-type ε3 allele.43 We did not observe an APOE effect on IL-9 levels in our series, but a larger cohort may be better powered to detect an interaction between these two factors.

We are not aware of a highly specific marker for Th9 cells, as flow cytometry for Th9 cells has relied on marker/cytokine combinations (eg, CD4+IL-9+IL-13−interferon-γ−IL-17−) not practical for large neuropathologic series. We thus examined multiple markers involved in Th9 differentiation and function in our neuropathologic analysis. In both racial groups, we found increased PU.1 transcript and protein levels in AD. This seems at first to contradict the race-specific changes in IL-9, but PU.1 is insufficiently specific for Th9 and found at even higher levels in regulatory T cells.44 Similarly, TGFBR2 (the receptor for TGF-β that regulates PU.1) expression also increased with AD regardless of race. TGF-β binding to this receptor in the presence of IL-4 is necessary for Th9 differentiation,45 but the exact in vivo stoichiometry between TGF-β and IL-4 is not known. We made the novel and important observation that AD was only associated with higher CSF IL-9 levels, higher brain TGFBR2/IL4RA ratio, and higher brain mast cell markers in African Americans. We propose that a higher ratio of surface TGF-β receptor to IL-4 receptor favors Th9 differentiation. Future experiments can confirm this hypothesis using single cell RNA-Seq analysis of CSF-derived T cells, as well as the clinically available IL-4 receptor blocker.46 If the observed alteration in IL-9 pathway proteins is related to slower cognitive decline observed in African Americans,47 manipulation of specific T-cell lineages using a US Food and Drug Administration–approved and well-tolerated agent may be an especially attractive therapy for AD in those without enhanced Th9 activity.

Our study was built on a large number of CSF samples from well-characterized middle-aged and older African Americans and Caucasians, IHC analysis in a second cohort, and transcriptomic analysis of a third cohort. It represents another step toward identifying biomarker and mechanism differences between individuals, but has some limitations. In keeping with reduced rates of participation in brain

donation among US minority groups,[48] the number of African Americans with available postmortem tissue for analysis—even if combined between the two cohorts—is limited. We have not yet correlated CSF biomarker levels with the corresponding gene polymorphisms in the coding or regulatory regions, and we did not focus on Th9 cells during our initial CSF flow cytometry work. Our CSF cohort consists mostly of people from the southern United States, where there is the greatest concentration of African Americans, and it is not clear how well these findings will generalize to African Americans from other US regions or native Africans. IL-9 variance was much greater in the brain than in the CSF, possibly reflecting different pools (eg, perivascular vs neuronal) of IL-9–immunoreactive cells. IL9 gene expression was itself not detected in the transcriptomic analysis, although some of its network partners were. The uneven distribution, stability, detection, and variance for genes and proteins implicated in the same regulatory pathways are not unique to AD and neuroinflammation, and warrant caution in interpreting unsupervised analyses without consideration for each network member. We tried to overcome some of these challenges through multiple cohorts and techniques, and demonstrated the most convincing data to date to support a different neuroinflammatory phenotype for AD in African Americans. Although the exact causes (genetic, environmental, cultural/behavioral) and consequences for baseline and AD-related IL-9 differences between African Americans and Caucasians need further investigation, this and similar studies reinforce the notion that not all AD-related changes derived in highly biased cohorts can be readily applied to real world populations.

Author Contributions

W.W., S.U., K.D.W., M.G., M.W.P., and W.T.H. contributed to study concept and design; all authors contributed to data acquisition and analysis; W.W., A.L.K., U.G., D.D.V., S.U., V.K., J.C.H., M.W.P., and W.T.H. contributed to drafting the manuscript and figures.

Potential Conflicts of Interest

W.T.H. consults for ViveBio, which manufactures lumbar puncture trays.

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