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Age-Dependent Alterations In Neuroimmune Signaling And Cognitive Vulnerability In HIV-1–Infected Humanized Mice

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Abstract :*Aging is a critical modifier of HIV-1–associated neuroimmune dysfunction, yet the mechanisms linking immune activation, neural injury, and functional vulnerability remain incompletely defined. This study investigated age-dependent neuroimmune alterations in HIV-1–infected humanized mice using integrated molecular, cellular, and behavioral assessments. A total of 60 humanized NOD/SCID/IL2Rγ-null mice were stratified into young (12–16 weeks) and aged (36–40 weeks) cohorts prior to HIV-1 infection and monitored for 20 weeks under suppressive antiretroviral therapy. Neuroinflammation was quantified by measuring human cytokine and chemokine profiles in brain tissue and cerebrospinal fluid using multiplex immunoassays, while microglial activation and synaptic integrity were evaluated through immunofluorescence and confocal microscopy. Cognitive and motor performance were assessed using novel object recognition and open-field tests. Compared with younger counterparts, aged HIV-1–infected mice exhibited significantly elevated levels of TNF-α, IL-6, and CCL2 in the brain (1.8–2.6-fold increase, $p<0.01$), accompanied by a 34% reduction in synaptophysin-positive synaptic density. Behavioral testing revealed a 29% decline in recognition index and increased locomotor variability in aged infected mice, despite comparable plasma viral suppression across age groups. Correlation analysis demonstrated that neuroinflammatory markers explained 47% of the variance in cognitive impairment independent of peripheral viral load. These findings indicate that aging amplifies neuroimmune dysregulation and functional vulnerability in HIV-1 infection, highlighting the need for age-sensitive strategies when evaluating neuroprotective interventions in HIV-associated neurological disease.*

Keywords: *HIV-1; aging; neuroimmune dysfunction; humanized mouse model; neuroinflammation; cognitive impairment*

1.INTRODUCTION

Substance use disorders (SUDs) remain a major public health concern in the United States and continue to contribute substantially to preventable morbidity and mortality. In recent years, overdose deaths, polysubstance use, and stimulant-related harms have

increased, indicating that the overall burden of SUD has not declined despite expanded public awareness and policy attention [1,2]. National surveillance systems consistently report that a considerable proportion of U.S. adults meet diagnostic criteria for SUD each year, yet only a minority receive any form of treatment, whether through specialty addiction services or general medical settings [3]. Emerging biomedical research further underscores the long-term neuroimmune and systemic consequences associated with substance exposure and comorbid conditions, highlighting the broader clinical significance of untreated SUD and its interaction with chronic disease processes [4]. This persistent gap between treatment need and utilization reflects ongoing structural and systemic limitations within the U.S. behavioral health system. Over the past decade, a growing body of research has examined trends in SUD treatment access and utilization. Analyses based on nationally representative survey data document modest increases in treatment uptake following insurance expansions and regulatory reforms, particularly for opioid use disorder (OUD) [5,6]. Expanded Medicaid coverage and the integration of medications for OUD into outpatient and primary care settings have been associated with improved access among certain populations [7]. During the COVID-19 pandemic, temporary telehealth policies reduced logistical barriers and supported treatment continuity for selected patient groups [8,9]. These policy changes demonstrate that structural adjustments can influence access to care, yet their effects appear uneven across demographic and socioeconomic strata. Despite these improvements, recent evidence indicates that treatment gains have not kept pace with rising SUD prevalence. Population-level analyses show that unmet treatment need remains substantial, particularly among younger adults, individuals with unstable income, and residents of rural areas [10]. Adults aged 18–25 consistently display lower treatment engagement despite high diagnostic prevalence, suggesting barriers beyond service availability, including stigma, limited perceived need, and weak linkage to care [11]. Rural communities continue to experience shortages of qualified providers and longer travel distances, reducing the likelihood of treatment initiation even when insurance coverage is present [12,13]. These findings point to persistent geographic and generational inequities in the distribution of behavioral health resources. Substantial disparities are also observed by insurance type and socioeconomic position. Individuals without stable insurance coverage or with lower household income are less likely to receive timely or continuous treatment, even after adjustment for clinical need [14]. Although racial and ethnic disparities in SUD treatment have been documented, findings vary by disorder type, service setting, and regional context, underscoring the importance of examining intersecting structural determinants rather than isolated characteristics [15]. Together, this literature suggests that treatment gaps arise from a complex interaction between demographic composition, socioeconomic constraints, insurance design, and local service capacity. Methodologically, most existing studies rely on regression-based approaches that estimate associations between individual characteristics and treatment receipt. While informative, such analyses do not quantify the relative contribution of each structural factor to observed treatment gaps at the population level. It therefore remains unclear whether persistent unmet need is primarily driven by shifts in population composition—such as age distribution or insurance coverage—or by changing relationships between these characteristics and access to care over time.

Decomposition techniques provide a framework to disentangle these components and have been widely applied in health disparities research [16]. However, their use in examining national SUD treatment gaps in recent U.S. data remains limited. Moreover, much of the recent literature focuses narrowly on OUD and medication-based treatment pathways. Although this focus is warranted given overdose trends, it does not fully capture the broader spectrum of SUD care, including services for alcohol and stimulant use disorders and treatment delivered outside specialty addiction settings [17]. A comprehensive assessment of treatment need and utilization across SUD types is necessary to understand how structural determinants shape overall access to care. The present study addresses these gaps by examining trends in SUD treatment need and treatment utilization among U.S. adults from 2012 to 2023 using nationally representative survey data. Annual weighted prevalence and age-standardized rates are estimated for both treatment need and treatment receipt to characterize temporal patterns. A multivariable decomposition framework is then applied to quantify the relative contributions of demographic, socioeconomic, insurance, and geographic factors to changes in treatment utilization and to persistent unmet need. By distinguishing between compositional shifts and structural differentials in access, this analysis provides a clearer understanding of the mechanisms underlying treatment gaps. The findings are intended to inform more targeted and equitable policy strategies aimed at strengthening the behavioral health system and improving SUD care delivery across diverse populations.

2.Materials and Methods

2.1 Animals and Study Population

This study included 60 humanized NOD/SCID/IL2Rγ-null (NSG) mice generated by transplantation with human $CD34^+$ hematopoietic stem cells. Animals were maintained under specific pathogen–free conditions with controlled temperature, humidity, and a 12 h light–dark cycle. Food and water were provided ad libitum. After immune reconstitution, mice were divided by age into a young group (12–16 weeks, n = 30) and an aged group (36–40 weeks, n = 30). Both male and female mice were included and distributed evenly across groups. All animal procedures were approved by the institutional animal care and use committee and followed established ethical guidelines.

2.2 Experimental Design and Control Groups

Following age stratification, mice underwent HIV-1 infection using a standardized intravenous protocol. Within each age group, animals were assigned to either an HIV-1–infected group treated with suppressive antiretroviral therapy (ART) or an uninfected age-matched control group. ART was initiated after confirmation of systemic infection and continued for 20 weeks to maintain viral suppression. This design allowed evaluation of age-related effects under comparable infection and treatment conditions. Uninfected controls were used to distinguish changes related to aging from those related to HIV-1 infection.

2.3 Measurements and Quality Control

Neuroimmune activity was measured by quantifying human cytokines and chemokines, including TNF-α, IL-6, and CCL2, in brain tissue and cerebrospinal fluid using multiplex immunoassays. Microglial activation and synaptic density were assessed by immunofluorescence staining for Iba1 and synaptophysin, followed by confocal microscopy and standardized image analysis. Cognitive and motor function

were evaluated using the novel object recognition test and the open-field assay. All measurements were performed by investigators blinded to group assignment. Technical replicates, internal controls, and calibration standards were included in each assay. Samples that did not meet predefined quality criteria were excluded from further analysis.

2.4 Data Processing and Model Specification

Biomarker values were log-transformed when necessary to improve normality. Group differences were analyzed using regression models adjusted for sex and experimental batch. For continuous outcomes, the primary model was specified as

$$Y_i=\beta_0+\beta_1 Age_i+\beta_2 Infection_i+\beta_3(Age_i\times Infection_i)+\varepsilon_i,$$

Where Y_i represents a molecular or behavioral outcome. To examine the association between neuroinflammation and cognitive performance, a linear model was defined as

$$C_i=\alpha_0+\sum_{k=1}^{K}\alpha_k I_{ik}+\gamma V_i+\eta_i,$$

where C_i denotes the cognitive score, I_{ik} represents inflammatory markers, and V_i indicates plasma viral load. Model fit was evaluated using adjusted R2R^2R2.

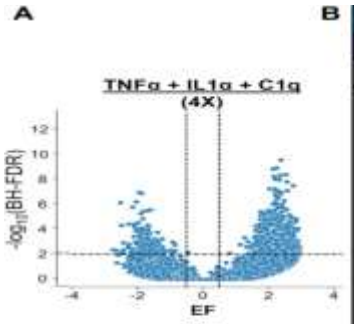
2.5 Statistical Analysis

All analyses were conducted using standard statistical software. Continuous variables are presented as mean ± standard error unless otherwise stated. Group comparisons used two-sided statistical tests. Associations between variables were assessed using Pearson correlation coefficients. Statistical significance was defined as $p < 0.05$. Sensitivity analyses were performed by excluding outliers and by re-estimating models without interaction terms to assess the stability of results.

3.Results and Discussion

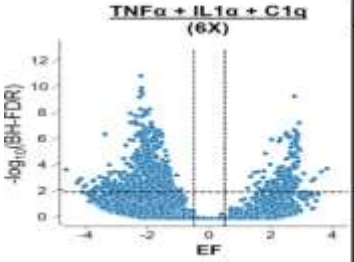
3.1 Aging amplifies central inflammatory signaling despite comparable viral suppression

During the 20-week period under suppressive antiretroviral therapy, young and aged mice showed similar levels of peripheral viral control, reducing the likelihood that group differences were driven by ongoing systemic viremia. In contrast, aged HIV-1–infected mice displayed a marked increase in brain inflammatory activity, with higher levels of TNF-α, IL-6, and CCL2 than younger infected mice. This finding is consistent with evidence that aging shifts myeloid cells toward a primed state with stronger cytokine output once inflammatory signaling is initiated [18,19]. Experimental studies further show that exposure to inflammatory cues alone can induce stronger transcriptional responses in glial cells, supporting the view that the aged central nervous system responds more strongly even when the initial stimulus is comparable. Fig.1 illustrates this high-gain response pattern reported in recent mechanistic work.



B

TNFα + IL1α + C1q – 4X			
Downregulated		Upregulated	
Gene	*E.F.*	*Gene*	*E.F.*
IKBKB-DT	-2.75	MTFR1	2.96
ENSG00000272715	-2.65	TTDN1	2.96
CTBP1-AS	-2.62	PTPN9	2.95
ENSG00000293175	-2.59	SLBP	2.95
ENSG00000279611	-2.56	SRRD	2.93
SIX2	-2.51	COMMD3	2.93
FAM167B	-2.49	DCUN1D5	2.92
SPTBN2	-2.48	MZT1	2.91
TNNC2	-2.43	IRF1	2.91
LRCOL1	-2.42	MED27	2.90



D

TNFα + IL1α + C1q – 6X			
Downregulated		Upregulated	
Gene	*E.F.*	*Gene*	*E.F.*
EEF1AKMT2	-4.65	JCHAIN	3.82
ENSG00000289138	-4.29	RIPK3	3.64
ENSG00000290450	-4.26	FAM87A	3.54
LMO2	-4.17	C11orf96	3.47
TMEFF1	-4.11	DAB1	3.42
FAM131A	-4.08	TRPC6	3.39
GEM	-4.01	IGSF23	3.35
NUP133	-3.98	COX14	3.35
PRECSIT	-3.95	NOG	3.34
GABPAP	-3.90	PTGDS	3.33

Figure 1.*Glial gene expression responses to inflammatory stimulation, showing increased astrocyte activation under HIV-1–related immune signaling.*

3.2 Microglial activation aligns with synaptic vulnerability in aged infection

Immunofluorescence analysis revealed stronger microglial activation in aged infected mice, together with reduced synaptophysin-positive synaptic density. This pattern suggests a shift from normal immune surveillance toward a tissue-stress state. The association between elevated cytokine and chemokine levels and synaptic marker loss supports a model in which sustained inflammatory signaling alters synaptic maintenance rather than producing only short-term functional effects. Previous studies on HIV-associated neurocognitive disorders describe multi-factor injury pathways in which inflammation, oxidative stress, and impaired glial support converge on synapses. A recent comprehensive review summarizes how host factors and environmental stressors shape these downstream injury processes and provides a broader framework for interpreting the present synaptic findings [20,21]. Fig.2 offers a representative example of this pathway-level view.

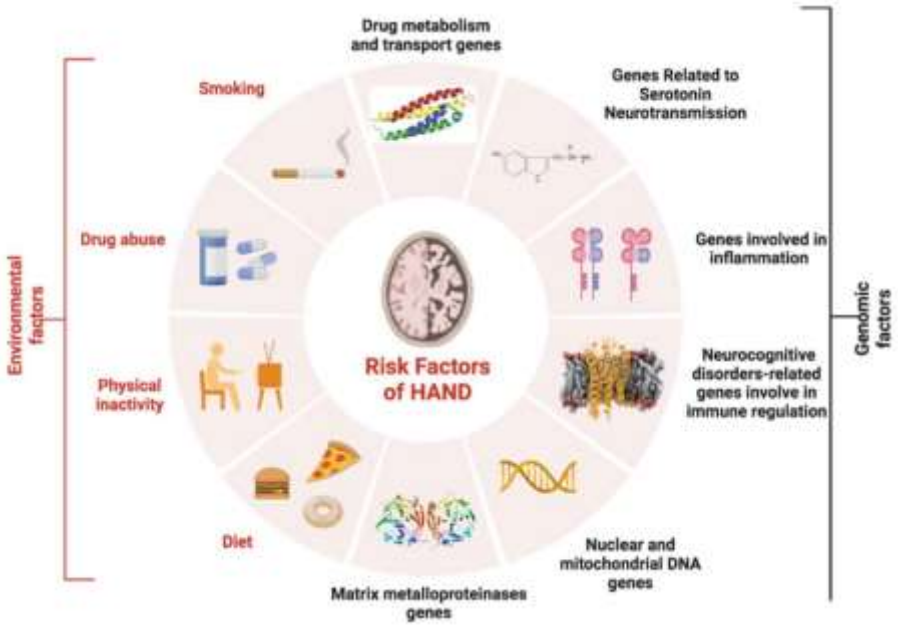


Figure 2.*Schematic overview of genetic, immune, and environmental factors contributing to synaptic damage and cognitive impairment in HIV-associated neurocognitive disorders.*

3.3 Behavioral deficits track neuroimmune markers more closely than peripheral indices

Behavioral assessments showed that aged infected mice had lower recognition performance and greater variability in locomotor activity compared with younger infected mice. Importantly, correlation analyses indicated that brain inflammatory markers accounted for a substantial proportion of cognitive variability independent of peripheral viral load. This finding argues against a simple explanation based on residual viremia. Instead, it supports clinical observations that neurocognitive symptoms can persist despite stable viral suppression and experimental evidence that immune activity within the central nervous system may remain elevated even when blood-based measures appear controlled [22]. Together, these results indicate that age functions as a biological modifier that alters how immune signals affect neural function, likely through interactions between glial activation, synaptic integrity, and reduced repair capacity.

3.4 Implications, comparison with prior studies, and remaining limitations

Overall, the results support a clear age-by-infection interaction in which aging raises the baseline inflammatory state of the central nervous system, increases the responsiveness of neuroimmune pathways, and heightens synaptic and cognitive sensitivity to inflammatory stress. Compared with recent mechanistic studies that focus primarily on glial polarization or inflammatory signaling in isolation, the present work strengthens interpretation by linking molecular markers, synaptic changes, and behavior within the same humanized model under antiretroviral therapy. Several limitations remain. The sample size limits detection of smaller effects and reduces power for stratified analyses such as sex-by-age interactions [23]. The experimental design is observational and cannot fully distinguish causal cytokine pathways from secondary responses. In addition, sampling from a single brain region may miss circuit-specific changes relevant to cognition. Future studies combining region-specific profiling, targeted manipulation of inflammatory pathways, and larger

cohorts will be needed to determine whether reducing neuroimmune activation can mitigate age-related cognitive vulnerability in treated HIV-1 infection.

4.Conclusion

This study shows that aging alters neuroimmune responses and increases cognitive vulnerability during HIV-1 infection, even when antiretroviral therapy maintains stable viral suppression. Using an age-stratified humanized mouse model, we found that older infected mice had higher levels of central inflammatory markers, greater loss of synaptic proteins, and worse cognitive performance than younger mice with similar peripheral viral control. By examining molecular, cellular, and behavioral outcomes together, the study clarifies how age-related immune changes in the brain are linked to synaptic integrity and function. These results extend existing research by identifying aging as a key modifier of HIV-associated neurological outcomes rather than a background factor. From a practical standpoint, the findings suggest that approaches aimed only at systemic viral control may be insufficient to protect brain function in older individuals, and that therapies targeting central inflammation and synaptic health may be needed. Several limitations should be noted, including the moderate sample size, the focus on a limited set of inflammatory markers, and analysis of selected brain regions, which may not capture all relevant pathways. Despite these limits, the study provides clear evidence that age-dependent neuroimmune mechanisms contribute to cognitive risk in treated HIV-1 infection and should be considered in future experimental and therapeutic strategies.

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