Journal of Neuroimmunology 381 (2023) 578141

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Viral co-infection, autoimmunity, and CSF HIV antibody profiles in HIV central nervous system escape

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ARTICLE INFO

Keywords:
HIV
Neuroinflammation
Programmable phage display
Neurosymptomatic escape
Asymptomatic escape
Metagenomic next-generation sequencing

ABSTRACT

Antiretroviral therapy (ART) suppresses plasma and cerebrospinal fluid (CSF) HIV replication. Neurosymptomatic (NS) CSF escape is a rare exception in which CNS HIV replication occurs in the setting of neurologic impairment. The origins of NS escape are not fully understood. We performed a case-control study of asymptomatic (AS) escape and NS escape subjects with HIV-negative subjects as controls in which we investigated differential immunoreactivity to self-antigens in the CSF of NS escape by employing neuroanatomic CSF immunostaining and massively multiplexed self-antigen serology (PhIP-Seq). Additionally, we utilized pan-viral serology (VirScan) to deeply profile the CSF anti-viral antibody response and metagenomic next-generation sequencing (mNGS) for pathogen detection. We detected Epstein-Barr virus (EBV) DNA more frequently in the CSF of NS escape subjects than in AS escape subjects. Based on immunostaining and PhIP-Seq, there was evidence for increased immunoreactivity against self-antigens in NS escape CSF. Finally, VirScan revealed several immunodominant epitopes that map to the HIV envelope and gag proteins in the CSF of AS and NS escape subjects. Whether these additional inflammatory markers are byproducts of an HIV-driven process or whether they independently contribute to the neuropathogenesis of NS escape will require further study.

1. Introduction

Infection of the central nervous system (CNS) begins early and continues throughout the course of systemic HIV-1 infection in the absence of anti-retroviral therapy (ART) (Valcour et al., 2012). In addition to its impact on systemic infection, ART also inhibits viral replication in the CNS, effectively eliminating detectable HIV from the cerebrospinal fluid (CSF) (Ostrowski et al., 2008; Canestri et al., 2010) and is effective in preventing the most severe direct CNS complication, HIV-associated dementia (HAD) (Grill and Price, 2014). While this CNS therapeutic success is the rule, there are unusual exceptions in which systemic replication is suppressed but remains at detectable levels in CSF. This is referred to as HIV CSF escape which is subdivided into three types: asymptomatic CSF escape (AS escape), neurosymptomatic CSF escape (NS escape) and secondary CSF escape (Ferretti et al., 2015).

Each of these three CSF escape syndromes is of pathobiological interest, but only NS escape has a clear, immediate clinical impact. AS escape has primarily been detected in cohort studies in which volunteers

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https://doi.org/10.1016/j.jneuroim.2023.578141
Received 14 February 2023; Received in revised form 12 June 2023; Accepted 20 June 2023
Available online 28 June 2023

undergo lumbar puncture (LP) as part of natural history studies of HIV infection, including treated infection (Mukerji et al., 2017; Edén et al., 2010; Edén et al., 2016). In contrast to AS escape, NS escape directly causes CNS injury. It presents with diverse neurological symptoms and signs, at times severe (Canestri et al., 2010; Peluso et al., 2012). CSF HIV RNA levels are usually higher than in AS escape, and neuroimaging is frequently abnormal (Peluso et al., 2012). The prevailing concept is that HIV encephalitis develops in these individuals because of some combination of i) reduced treatment adherence and ii) local drug resistance (Mukerji et al., 2017).

Since HIV CSF escape is rare, the question has arisen whether other, yet-undefined factors may predispose to or provoke NS escape, including the presence of other pathogens such as Epstein-Barr virus (EBV) or cytomegalovirus (CMV) (Ferretti et al., 2015; Lupia et al., 2020) or a systemic or CNS autoimmune process, with its own potential for injury. In order to investigate these possibilities, we interrogated CSF samples from NS escape and AS escape subjects. Metagenomic next-generation sequencing (mNGS) (Ramachandran and Wilson, 2020) and pan-viral antibody profiling using programmable phage display (VirScan) (Xu et al., 2015; Schubert et al., 2019) were used to identify other infectious agents in an unbiased manner. We looked broadly for autoantigens using rodent brain tissue-based immunofluorescence and pan-human proteome phage immunoprecipitation sequencing (PhIP-Seq) (Larman et al., 2011; O'donovan et al., 2020).

2. Materials and methods

2.1. Subject enrollment

The samples used in this case-control study were collected between 2000 and 2018 in the context of HIV research studies at several medical centers: Sahlgrenska University Hospital, Gothenburg, Sweden and Zuckerberg San Francisco General Hospital, University of California San Francisco, San Francisco, CA, USA (Gisslen et al., 2021). The focus of this study is on NS escape ($n = 32$) and AS escape ($n = 19$) with non-HIV infected controls ($n = 6$). NS escape, AS escape, and non-HIV infected control samples all came from unique individuals sampled at a single time point. A broad comparator group of people living with HIV (PLWH) was included as background for PhIP-Seq analyses, including some longitudinal samples taken at different time points from the same patient. The salient clinical characteristics of these PLWH are described in supplementary table 2 and are stratified by clinically relevant groupings.

2.2. mNGS for pathogen detection

DNA sequencing libraries were prepared using a previously described protocol optimized and adapted for miniaturization (Ramachandran et al., 2022), and libraries were sequenced on a NovaSeq 6000 machine (Illumina) to generate 150 nucleotide (nt), paired-end reads. Candidate DNA virus pathogens were identified from raw mNGS sequencing reads using CZID v3.2, a cloud-based, open-source bioinformatics platform designed for detection of microbes from mNGS data (Kalantar et al., 2021).

2.3. VirScan (pan-viral) and peptidome (pan-human proteome) serologic assays

PhIP-Seq assays to identify anti-viral antibodies (i.e., VirScan) and autoantibodies were performed as previously described in technical replicate (Schubert et al., 2019; Song et al., 2021). Briefly, phage display libraries were amplified in *E. coli* and incubated overnight with 2uL of subject CSF or serum and underwent two rounds of immunoprecipitation. Barcoded phage DNA were then sequenced on a NovaSeq 6000 (Illumina).

2.4. Bioinformatic analysis of PhIP-Seq data

For the VirScan and human peptidome PhIP-Seq analyses, data were analyzed using previously described methods (Schubert et al., 2019; Song et al., 2021) (Supplemental Methods). Briefly, peptide sequences were aligned to their respective reference library, and counts were normalized by creating a ratio of specific peptide reads to the number of sequencing reads and multiplying by 100,000 to create a measure of reads per hundred thousand (rpK). rpKs for individual peptides in each subject were filtered using a cutoff fold-change (FC) of >10 above the mean background rpK generated from null IPs. Additional analysis was performed for the human peptidome assay (Supplemental Methods). Peptide position mapping was performed by aligning peptide sequences in R Studio using the msa (multiple sequence alignment) Bioconductor package (Bodenhofer et al., 2015) and using the envelope protein and gag/pol polyprotein portions of the HXB2 HIV clade B sequence (GenBank K03455.1) as reference sequences.

2.5. Anatomic mouse brain staining

Mouse brain sections fixed overnight in 4% paraformaldehyde were immunostained with CSF at 1:25 dilution were prepared as previously described (Song et al., 2021) (Supplemental Methods). CSF immunostaining was independently assessed by two scientists (SJP and WB) experienced in immunohistochemistry analysis in a blinded manner.

2.6. Statistical methods

All data comparisons between two subsets of subjects were made using the Mann-Whitney test or Chi-square test, and exact n and error bars are provided and defined in figure legends. All comparisons involving two or more subsets of subjects were performed with the ANOVA test. Sample sizes were determined by sample availability and not a statistical power calculation.

3. Results

3.1. Features of the study cohort

This study included AS escape ($n = 19$), NS escape ($n = 32$) subjects as well as HIV-negative controls ($n = 6$) (Fig. 1a). The salient HIV-related background characteristics of the study cohort are summarized in Table 1. The HIV CSF escape groups adhered to recent definitions (Winston et al., 2019), and the samples were obtained in the context of clinical presentation (NS escape). Subjects enrolled as part of cohort studies (all other subjects) and have been described previously (Gisslen et al., 2021; Hermansson et al., 2019; Peterson et al., 2014).

3.2. CSF mNGS detects a higher burden of EBV DNA in NS escape subjects

We sequenced 45 CSF samples from AS and NS escape subjects using DNA mNGS with a median sequencing depth of 41,163,352 reads per sample (IQR 16,384,312-133,365,998 reads per sample). 7 (NS escape $n = 1$ and AS escape $n = 6$) samples included in other experimental assays in this paper were not included due to limited sample volume. There was a statistically significant increase in EBV detection by CSF mNGS in the NS escape subjects ($n = 13$ out of 32) when compared to AS escape subjects (n = 1 out of 13) (Chi-square test, $p < 0.05$) (Fig. 1b). In addition to EBV detection (0.1–0.8 reads per million (rpM)), we detected CMV and human herpes virus-8 (HHV-8) in one NS escape subject and HHV-6 in another NS escape subject (Supp Table 1). Aside from human herpes viruses, no other neuroinvasive viruses were detected in any of the 45 subjects.

Using VirScan, AS escape subjects had a significantly higher average enrichment of EBV-specific peptides relative to NS escape subjects

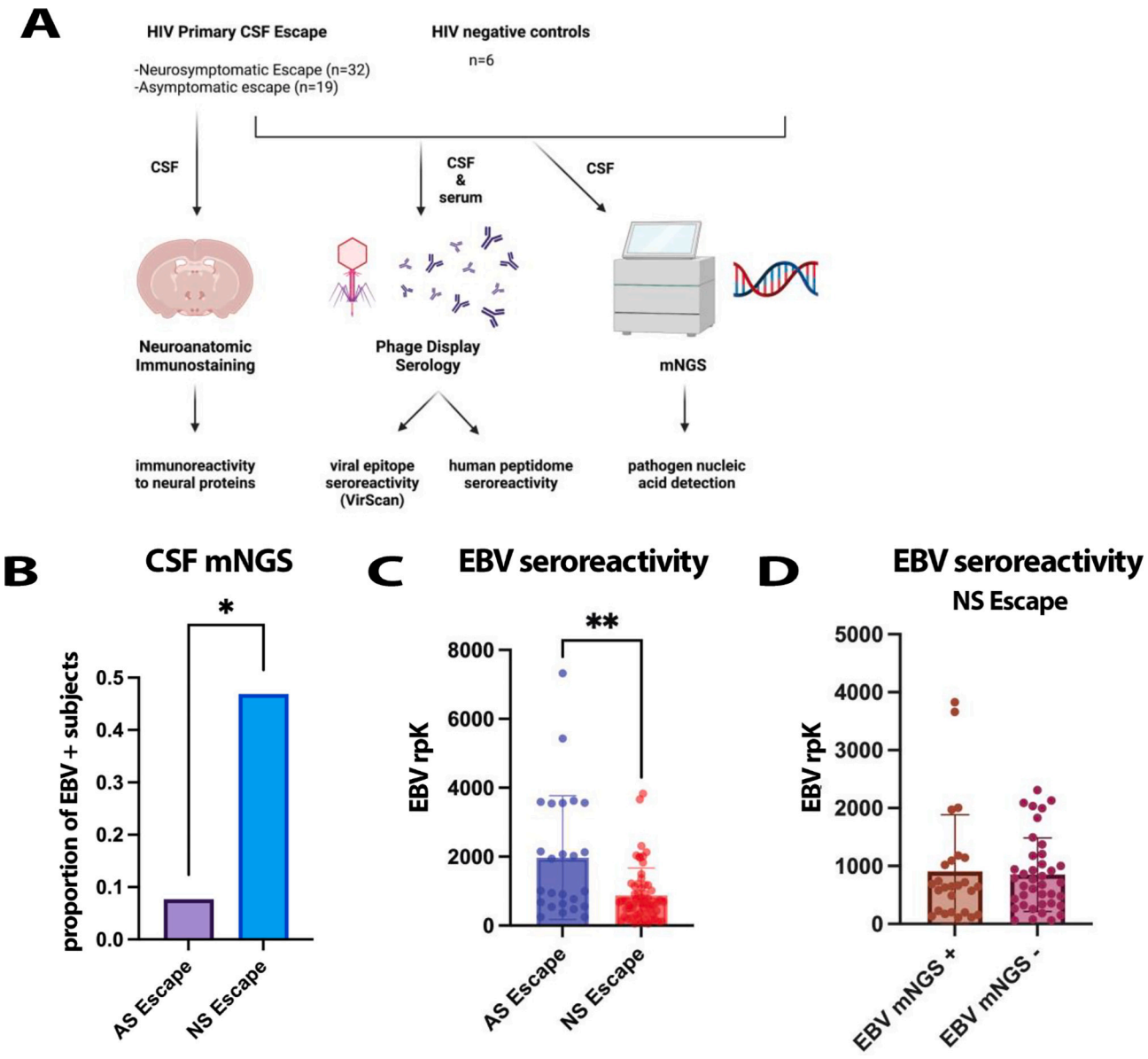

Fig. 1. CSF mNGS detects a higher burden of EBV in NS Escape subjects A.) Schematic of the study design. PLWH with diverse neurologic phenotypes, including NS and AS escape, underwent a lumbar puncture. CSF autoantibody and anti-viral antibody profiling was done using phage display assays in technical replicate (human peptidome and VirScan) and neuroanatomic staining on rodent brain tissue. Unbiased metagenomic sequencing was done on DNA extracted from CSF. B.) Proportion of subjects with Epstein-Barr virus (EBV) detected by CSF mNGS in NS escape ($n = 32$) and AS escape ($n = 13$) subjects.C.) Quantification of EBV seroreactivity by VirScan in AS and NS escape subjects (in replicate). Results are reported in reads per 100,000 (rpK). D.) Quantification of EBV seroreactivity by VirScan (performed in replicate) in NS escape subjects who had EBV detected in their CSF by mNGS versus those where EBV was not detected by mNGS. Statistical analyses were done using a Mann-Whitney *U* test in panels C and D. Statistical analysis for panel B was done using a chi-square test. Data represents mean rpK +/− SEM.

(Fig. 1c). Relative to AS escape subjects, the CSF of NS escape subjects did not differentially enrich viral peptides in non-retroviridae/non-herpesviridae viral families (Supp. Fig. 1b). There was no difference in enrichment for EBV-specific peptides in NS escape subjects for whom EBV nucleic acid was detected in the CSF versus those in whom EBV was not detected (Fig. 1d). Additionally, we examined whether EBV rpM as measured by DNA mNGS correlated with HIV CSF viral load and found that there was no correlation between these two variables (Supp. Fig. 2a-b).

3.3. VirScan shows decreased HIV envelope protein enrichment in subjects with NS escape compared to AS escape

In peripheral HIV infection, the humoral immune response is primarily directed against the HIV envelope protein and the gag/pol polyprotein (Moir and Fauci, 2017). In our cohort, VirScan identified two immunogenic regions in the envelope protein starting at amino acid (AA) positions 289 and 577 from the CSF of both AS and NS escape subjects (Fig. 2a). The AA 289 epitope maps to the V3 loop of the envelope protein, which is an important determinant of X4/R5 tropism of viral quasi-species (Cardozo et al., 2007). The epitope at AA 577 maps to the C-terminal heptad repeat 2 region (CH2 terminal), which plays an important role in CD4 receptor binding (Joseph et al., 2018). We found that CSF immunoglobulins enriched HIV envelope peptides from AS escape subjects ($n = 19$) to a greater degree than NS escape subjects ($n = 32$) (AS escape median 64,567 rpK IQR 56,306–69,994 rpK vs NS escape median 35,734 rpk IQR 22,536–47,053 rpK) ($p < 0.001$) (Fig. 2b). This difference is largely driven by an enrichment of the AA 577 CH2 epitope

Table 1
Background characteristics of the subject groups.

SUBJECT CLASSIFICATION	Total patients	Total CSF samples	Sex (M:F)	Age	Blood T lymphocytes			CSF	Plasma	CSF
					CD4+	CD8+	Nadir CD4+	WBCs	HIV RNA	HIV RNA
				years	cells/μL			cells/μL	copies/mL	copies/mL
Main Groups										
HIV negative controls	6	6	5:1	46.9	751.5 (697.3–813.3)	308.5 (232.8–422.5)	NA	2.5 (1.3–3)	NA	NA
HIV CSF Escape										
Asymptomatic Escape	19	19	12:9	49	412 (330–680)	860 (720–1100)	145 (93.25–200)	2 (0.5–4)	19 (19–19)	80 (59.5–165.25)
Neurosymptomatic Escape	35	35	22:13	46	450 (343.75–615)	800 (595–935)	67 (30.25–176.75)	20 (10.5–32)	89 (19–385)	1648 (603–5253.25)

statistics in this table are reported as median and IQR (if appropriate).
NA: not applicable.
Nadir CD4 counts in untreated were at or near counts at visit.

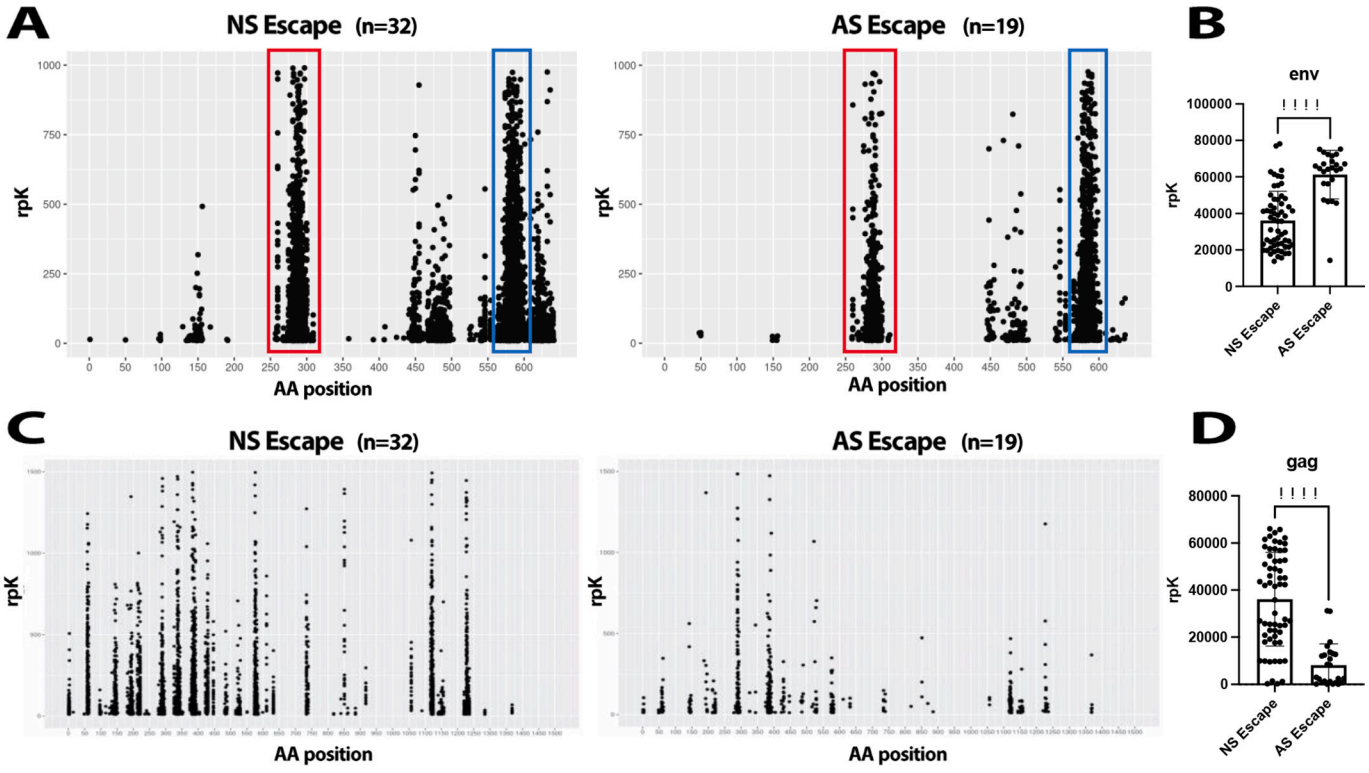

Fig. 2. Deep epitope profling of HIV antigenic specifcity in the CSF reveals differential epitope targeting between AS and NS escape subjects A.) CSF antibody epitope maps of the HIV env protein from AS and NS escape subjects. Each point on the graph represents the targeting of a single peptide from a single subject. Epitopes of note at AA position 289 (red) and AA position 577 (blue) are highlighted B.) Quantifcation of the summed rpK of peptides mapping to the env protein enriched by CSF samples from subjects with AS and NS escape. C.) CSF antibody epitope maps of the HIV gag protein from AS and NS escape subjects. Each point on the graph represents the targeting of a single peptide from a single subject D.) Quantifcation of the rpK of peptides mapping to the gag protein enriched by CSF from subjects with AS and NS escape. All statistical analyses were done using a one way ANOVA test. Data represents mean rpK +/− SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in AS escape subjects.

3.4. VirScan shows increased antibody reactivity to the gag protein in subjects with NS escape compared to AS escape

In contrast to our envelope protein findings, epitope mapping of the gag protein across both AS and NS escape subjects shows that antibody specificity was much more diffuse (Fig. 2c). However, we found that there was a significant enrichment of HIV gag peptides in NS escape (median rpK 41,770, IQR 20,493–54,051 rpK) compared to AS escape cases (median rpK 2953, IQR 673–12,848 rpK) ($p < 0.001$) (Fig. 2d). The difference in HIV gag protein enrichment in NS escape subjects was largely driven by peptides starting at AA position 433, mapping to the gag spacer peptide 2 (p1) domain, which is a recently discovered, protective T-cell epitope (Yang et al., 2021).

3.5. CSF from some NS escape subjects demonstrate immunoreactivity to rodent brain tissue

Of the 32 NS escape cases whose CSF was immunostained, 10 were classified as positive (Fig. 3a). In contrast, only one case (case 66) out of 19 in the AS escape group was classified as positive. There were some commonalities in the brain regions that demonstrated staining (Fig. 3b-c).

3.6. NS escape subjects show increased number of candidate autoantibodies in the CSF by PhIP-Seq

NS escape subjects had significantly higher numbers of candidate CSF autoantigens (median: 39 genes enriched, IQR 23–54) than AS escape subjects (median: 23 genes enriched, IQR 11.5–32) ($p < 0.0001$) (Fig. 4b). While we did not find evidence of a shared or "public" autoantigen in NS escape subjects, peptides from CNS associated proteins were enriched across multiple NS escape subjects, including SZT2 ($n = 4$) with an average FC of 239.8, ROBO2 ($n = 2$) with an average FC of

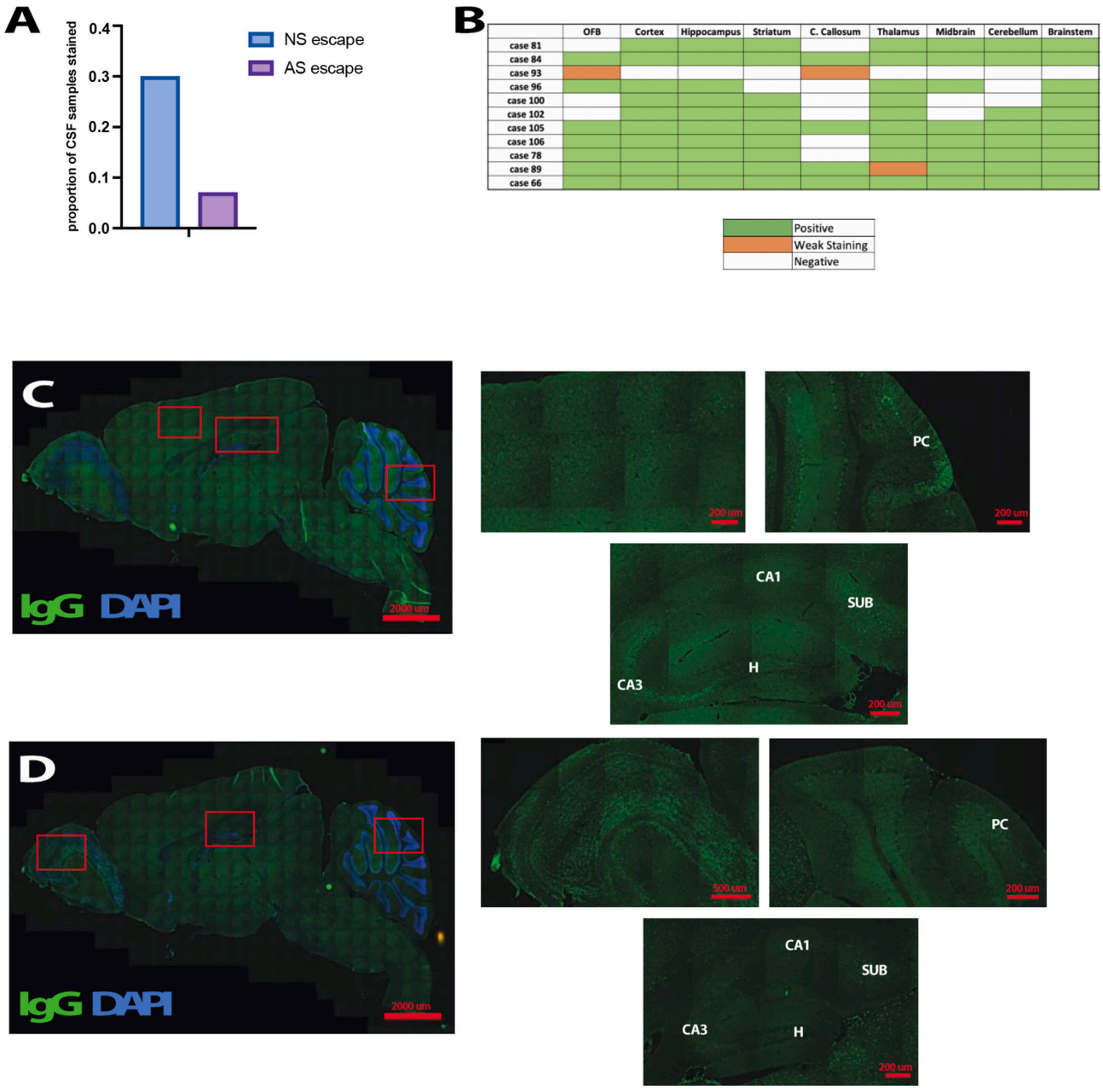

Fig. 3. Increased neuroanatomic immunoreactivity in NS escape CSF suggests increased prevalence of anti-neural antibodies A.) Quantification of proportion of NS escape (n = 32) and AS escape (n = 13) subject CSF that had a positive staining pattern on the anatomic staining platform. B.) Matrix indicating brain regions with anatomic immunoreactivity of NS and AS escape CSF C.) Whole brain AxioScan image of a sagittal slice of brain tissue stained at a 1:25 dilution with CSF (green) from NS escape subject case 105 and the nuclear stain DAPI (blue). Additional high-resolution representative images are shown from cortex (top left) - note the cytoplasmic labeling of neurons in multiple cortical layers, cerebellum (top right) - note the labeling of neurons in the pukinje cell layer (PC) and hippocampus in CA3, the hilus (H) and subiculum (SUB) (bottom). D.) Whole brain AxioScan image of a sagittal slice of brain tissue stained 1:25 with patient CSF from NS escape patient case 106. Additional high-resolution representative images are shown from olfactory bulb (top left) cerebellum - note staining of neurons in the Purkinje cell layer (PC) (top right) and hippocampus - note labeling of scattered neurons in the pyramidal fields and hilus which are likely to be interneurons (bottom). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

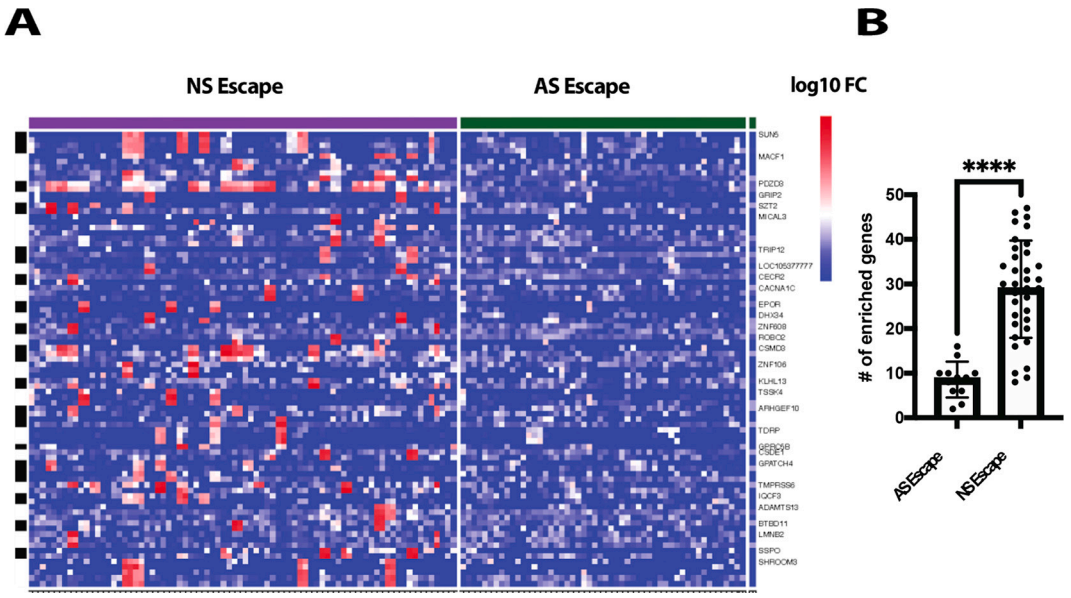

Fig. 4. NS escape CSF shows increased autoantibody prevalence by PhIP-Seq CSF from AS and NS escape subjects was run on a pan-human peptidome serology assay. Proteins were considered candidate autoantigens if they had at least one peptide enriched relative to a null template AG bead control and non-HIV CSF escape background (see methods) A.) Heatmap of genes in AS ($n = 19$) and NS escape (n = 32) cases that were enriched above background. Each column is a single subject sample (grouped by NS and AS escape) and each row is a single enriched peptide (grouped by gene) B.) Quantification of the number of genes in AS and NS escape cases that were enriched above background. All statistical analyses were done using a Mann-Whitney U test. Data represents mean rpK +/− SEM.

152.6, ARSA (n = 2) with an average FC of 30.42, and MACF1 ($n = 8$) with an average FC of 227.4.

4. Discussion

Neurological symptoms usually abate in NS escape subjects when ART is adjusted, paralleled with decreased CSF HIV RNA concentrations (Mastrangelo et al., 2019). This provides strong evidence for HIV-1 as the etiologic agent. However, there are HIV subjects with increased HIV replication in the CSF with no neurological symptoms (AS escape) (Edén et al., 2016), calling into question whether the pathogenesis of NS escape can be solely explained by CNS HIV replication. Here, we deployed a number of tools comparing AS and NS escape subjects to determine whether there was evidence for a neuroinvasive co-infection and/or autoimmunity in NS escape.

With regard to potential co-infections, we detected EBV DNA in the CSF at significantly higher rates in NS escape subjects compared to AS escape subjects using mNGS. Especially given the beneficial response that NS escape subjects have to ART alone and not to other antiviral therapies, the increased detection of EBV DNA in the CSF of NS escape subjects likely indirectly reflects a more inflammatory milieu rather than EBV playing a direct role in pathogenesis. Indeed, this finding could merely reflect an increased B-cell abundance in NS escape samples, made more plausible by the fact that, as is typical in NS escape, our NS escape cohort had a higher CSF white blood cell count than the AS escape patients (Table 1). This finding is also consistent with studies showing that EBV positivity in PLWH correlates with higher HIV RNA levels and CNS inflammation (Lupia et al., 2020; Kleines et al., 2014). As a complement to mNGS, we utilized pan-viral antibody profiling of CSF with VirScan. We found that, paradoxically, AS escape subjects displayed increased enrichment of EBV-specific peptides compared to NS escape subjects. Since VirScan is only semi-quantitative and broadly detects both neutralizing and non-neutralizing anti-viral antibodies, it remains an open question whether this decrease in EBV peptide enrichment in NS escape subjects actually reflects a less effective humoral response to EBV.

We found two immunodominant HIV envelope protein epitopes in both the CSF and in the serum that map to AA position 289 in the V3 region and AA position 577 in the CH2 terminal region. The V3 region is integrally involved in both receptor binding and the determination of viral tropism (Joseph et al., 2015; Gorry et al., 2002). The CH2 terminal region plays a key role in HIV-1 entry by membrane fusion (Mzoughi et al., 2019). When we compared the enrichment of these envelope peptides between NS and AS escape subjects, we found that AS escape subjects more highly enriched the CH2 terminal epitope relative to NS escape subjects. The difference between the CSF HIV envelope immunoreactivity profiles of NS and AS escape subjects awaits confirmation with more quantitative serologic tests (e.g., enzyme-linked immunosorbent assay) and in independent patient cohorts. If confirmed, differences in the CNS anti-HIV envelope protein antibody response in NS and AS escape would merit further investigation as a potential contributor to, or marker of, disease phenotype.

In addition to these conserved envelope peptides, we also found a gag peptide at AA position 433 that was significantly enriched in NS escape subjects relative to AS escape subjects. This peptide maps to the gag spacer peptide 2 (p1) domain, which is a recently discovered, protective T-cell epitope (Yang et al., 2021). Notably, all three of these epitopes (env AA positions 289 and 577, gag AA position 433) were also identified by Eshleman et al (Eshleman et al., 2019) in their VirScan study profiling anti-HIV antibodies in sera from 57 PLWH. Their study identified 4 antigens that were associated with disease duration, and three of those four epitopes are contained within the V3 and CH2 terminal regions we identified as well as the gag epitope that was enriched in NS escape subjects.

HIV subjects can exhibit broad B-cell dysfunction even in the presence of ART (Moir and Fauci, 2017). Here, we found that the CSF of NS escape subjects demonstrated an increased rate of rodent brain immunoreactivity when compared to AS escape subjects, suggesting an increased burden of anti-neural antibodies. Additionally, PhIP-Seq identified candidate CNS-associated autoantigens, including SZT2, ROBO2, ARSA, and MACF1 across multiple NS escape subjects. Interestingly, MACF1 has been shown to regulate microtubule and actin dynamics in developing neurons, and mutations in this gene have been associated with lissencephaly (Dobyns et al., 2018). These results suggest an increased burden of CNS autoimmunity in NS escape subjects when compared to their AS escape counterparts. In light of recent

literature documenting cases of autoimmune encephalitis and a steroid responsive, CD8+ T cell encephalitis in the setting of chronic HIV infection, including in cases of HIV escape, these findings raise the possibility that CNS autoimmunity may play a role in some cases of NS escape (Moloney et al., 2020; Patarata et al., 2016; Cunill et al., 2016; Pinzon-Charry et al., 2019; Bartley et al., 2023; Shenoy et al., 2023). However, it is unclear whether the differences we identified are a cause or a consequence of NS escape, especially given that many of the candidate autoantigens are intracellular proteins, and as a result, antibodies targeting them are likely not pathogenic.

This study has a number of limitations. While we identified a higher number of NS escape subjects versus AS escape subjects with EBV DNA in CSF, we cannot rule out the possibility of other co-infections present in NS escape subjects. Because we performed mNGS on CSF DNA alone, owing to RNA degradation in some samples, we were insensitive to detecting neuroinvasive RNA viruses. As implemented, the VirScan assay preferentially immunoprecipitated IgG and not IgM antibodies, making the assay less sensitive to an acute humoral anti-viral response. In addition, these subjects were immunosuppressed and may have had a blunted humoral immune response. Additionally, antibodies targeting conformational or post-translationally modified antigens are not well detected by the mostly linear peptide VirScan and human peptidome assays. Furthermore, these studies were performed on CSF that in some cases was over two decades old and had undergone multiple freeze thaws, raising the potential for sample degradation. Finally, our candidate autoantigens need to be validated using orthogonal assays and assessed in prospective cohorts to determine whether they might serve as biomarkers of disease and/or contribute to disease pathogenesis.

We deployed agnostic tools in a population of AS and NS escape subjects to assess for evidence of a neuroinvasive co-infection and/or autoimmunity that might enhance our understanding of HIV escape syndromes. In the CSF, we more frequently detected EBV DNA and immunoreactivity to self-antigens in NS escape subjects compared to AS escape subjects. We also described potential differential HIV antibody responses in the CSF of AS and NS escape patients. While preliminary, this study provides multiple fruitful areas for future inquiry into the underlying the pathophysiology HIV CNS escape.

Funding

Supported by grant from NIH R01 R01NS094067 and 3R01NS094067-05S1 to Dr. Richard W Price and colleagues and R01MH122471 (SJP, MRW, JLD), K08NS107619 (MRW) and Westridge Foundation (MRW and IAH). CM Bartley was supported by a Hanna H. Gray Fellowship, Howard Hughes Medical Institute, a President's Postdoctoral Fellowship Program, the University of California, and the John A. Watson Scholar Program, UCSF.

Contributions

Hawes IA: conceptual design, experimental design, PhIP-Seq experiments, mNGS data analysis, PhIP-Seq data analysis, data interpretation, writing

Alvarenga B: PhIP-Seq assay

Browne W: rodent brain anatomic immunostaining, confocal microscopy

Wapniarski A: mNGS library preparation and sequencing

Dandekar R: PhIP-Seq analysis

Bartley C.M.: PhIP-Seq analysis

Sowa G: PhIP-Seq analysis

Pleasure S: rodent brain anatomic immunostaining analysis, supervising

DeRisi J: supervising, writing

Dravid A.N.: clinical data collection

P. Cinque: clinical data collection

Gisslen M: clinical data collection and analysis

Price RW: initiation and conceptual study design, clinical data collection and analysis, writing

Wilson MR: conceptual study design, experimental design, data interpretation, writing

Declaration of Competing Interest

Dr. Joseph DeRisi has received grants from the Chan Zuckerberg Biohub, personal fees from the Public Health Company and from Allen & Company; Dr. Michael Wilson has received unrelated grants from Roche/Genentech and Novartis as well as speaking honoraria from Novartis, Takeda, WebMD and Genentech. Drs. Wilson and DeRisi serve as co-founders and advisors for Delve Bio. Dr. Christopher Bartley has received an honorarium for speaking to the Commonwealth Club and owns shares in NowRx Inc. M. Gisslen has received research grants from Gilead Sciences and Janssen-Cilag and honoraria as speaker, DSMB committee member and/or scientific advisor from Amgen, AstraZeneca, Biogen, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline/ViiV, Janssen-Cilag, MSD, Novocure, Novo Nordic, Pfizer and Sanofi, all unrelated to the content of this manuscript.

Data availability

Data will be made available on request.

Acknowledgements

Sequencing was performed at the UCSF Center for Advanced Technology, supported by UCSF PBBR, RRP IMIA, and NIH 1S10OD028511-01 grants. We thank Thomas Ngo for his guidance on the rodent brain anatomic immunostaining platform and the patients and their families for their participation in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2023.578141.

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