Circulating class-switched antibody-secreting cells are enriched among MS patients self-reporting 'Black African', or 'Latin American/Hispanic' ethnicity relative to those reporting 'Caucasian' ethnicity

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Study Rationale

Ethnicity-associated clinical disparity in MS is well documented.[1,2] In the United States, MS patients self-reporting with 'Black African', or 'Latin American/Hispanic' ethnicities (BALA) are more likely to experience a severe disease course relative to MS patients self-reporting 'Caucasian/European' ethnicity (CA). Socioeconomic status modulates but does not completely account for this disparity.[3]

Despite numerous reports corroborating these trends over the past 16 years, there are no published studies (to our knowledge) that directly investigate underlying immunobiology between these patient populations. Retrospective chart review demonstrates heightened intrathecal IgG among African American MS patients relative to Caucasian patients.[4,5]

This observation, alongside the established role for B cells in MS pathogenesis,[6,7,8] prompted our **hypothesis;** individuals with MS self-identifying with ethnicity groups more likely to experience heightened clinical severity will exhibit greater circulating antibody-secreting cell (ASC) levels

We therefore conducted the following prospective, cross-sectional study employing well-established techniques to directly quantify the relative levels of circulating antibody-secreting cells in MS patients across different ethnic group cohorts.

Approach: Study population & technical methods

Recruitment: Convenience sample population from Weill Cornell Multiple Sclerosis Center or local community. All study subjects were recruited & consented according to Weill Cornell Medicine Institutional review board-approved protocol#1508016490R003.

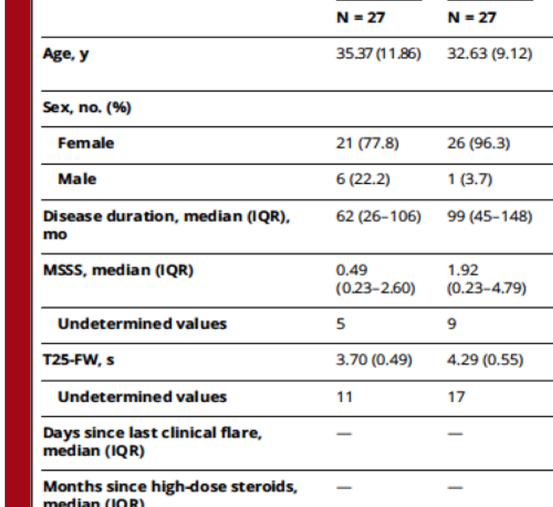
Self-reported identity survey: Consenting study participants reported ethnicity according to one or more categories: 'Black African'; 'Latin American/Hispanic'; 'Caucasian/European'

Study cohorts: Individuals with MS or age-matched healthy donors, grouped into one of two ethnicity cohorts delineated based on precedent for heightened MS severity.

- ***Natalizumb-treated*** (NAT) - 54 participants (27 BALAwMS NAT, 27 CAwMS)
- ***Off treatment subjects*** (No DMT) - 20 participants that were off of drug at the time of study draw (12 BALAwMS, 8 CAwMS)
- ***Healthy donor subjects*** (HD) - Lacked MS Dx (11 BALA HD, 13 CA HD).

Table 1: Demographic and clinical characteristics of study participants

	MS NAT			MS no DMT			HD		
	CA	BALA		CA	BALA		CA	BALA	
	N = 27	N = 27	*p* Value	N = 8	N = 12	*p* Value	N = 13	N = 11	*p* Value
Age, y	35.37 (11.86)	32.63 (9.12)	0.35	34.88 (6.10)	37.17 (6.66)	0.45	29.33 (6.97)	32.82 (8.85)	0.30
Sex, no. (%)			0.1			0.17			0.36
Female	21 (77.8)	26 (96.3)		3 (37.5)	9 (75.0)		2 (15.4)	4 (36.4)	
Male	6 (22.2)	1 (3.7)		5 (62.5)	3 (25.0)		11 (84.6)	7 (63.6)	
Disease duration, median (IQR), mo	62 (26–106)	99 (45–148)	0.23	3 (0–70)	56 (13–132)	0.12	—	—	—
MSSS, median (IQR)	0.49 (0.23–2.60)	1.92 (0.23–4.79)	0.28	1.16 (0.89–2.64)	1.86 (0.59–5.90)	0.85	—	—	—
Undetermined values	5	9					—	—	—
T25-FW, s	3.70 (0.49)	4.29 (0.55)	0.009	3.85 (1.06)	5.30 (1.92)	0.35	—	—	—
Undetermined values	11	17		6	5		—	—	—
Days since last clinical flare, median (IQR)	—	—	—	146 (52.5–364.5)	56 (23–659)	0.90	—	—	—
Months since high-dose steroids, median (IQR)	—	—	—	8.80 (4.20–73.85)	3.30 (1.60–11.00)	0.35	—	—	—
None	—	—	—	4	3		—	—	—



ASC Gating General Antibody-secreting cell gate using CD19, CD27 and CD38. (A) Circulating ASC population largely lacked CD20, while expressing moderate to high amounts of CD86 and HLA-DR, as indicated in the fluorescence minus one gates in panel B.

Technical workflow & ASC gating

1. Fresh-drawn whole blood from WCM MS Center or otherwise local convenience sample population.
2. Isolated peripheral blood mononuclear cells (PBMCs) through density gradient ficoll centrifugation.
3. Ficoll-spun buffy coats harvested within hours of peripheral blood draws and resuspended in volumes of standard staining buffer equal to the original whole blood volume.
4. Standard flow cytometry analysis using B cell/ASC markers: CD19, CD20, CD86, CD27, CD38, CD138, HLA-DR, IgM, IgD
   - ASC frequency
   - ASC event count

Results

Figure 1. Subjects with MS of Black African or Latin American self-identity exhibit enhanced ASC frequencies over those of Caucasian identity.

(A) Representative gating strategy for ASCs; $CD27^{hi}$ $CD38^{+}$ total ASCs were selected from CD19+ cells for downstream phenotypic subset analysis. (B) Representative gates for class-switched IgD^{-} $CD27^{+}$ total ASCs and $CD38^{+}$ $CD138^{+}$ subpopulation. (C) Representative gates for IgM^{+} ASCs. (D and E), Average frequencies of previously described class-switched (D) and IgM^{+} (E) ASC populations; error bars represent SD, p values determined the by 2-sided t test.

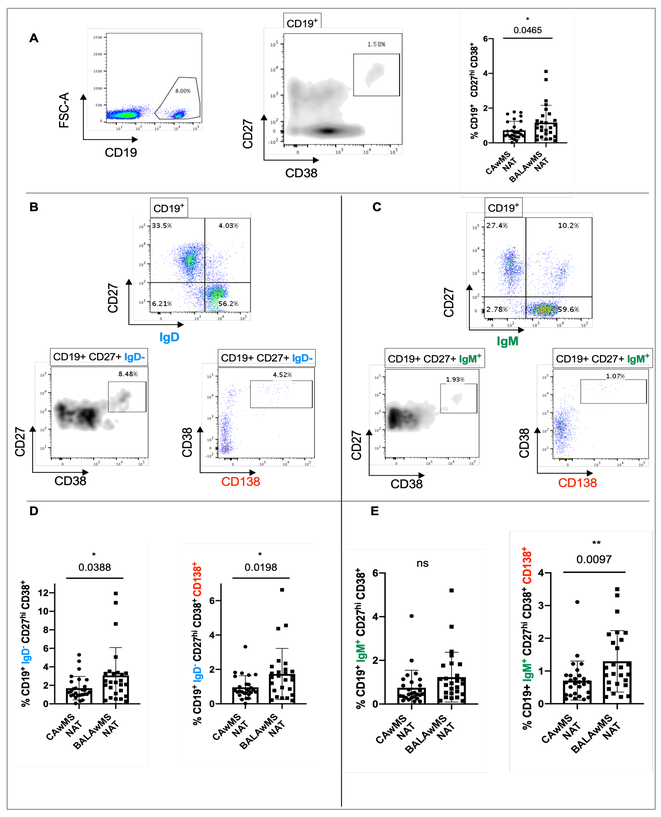


Figure 2. Self-identity–based differential ASC frequency is present among those with MS and not HDs.

Average frequencies of previously described total $CD19^{+}$(A), class-switched $CD19^{+}IgD^{-}$ (B and D) and unswitched $CD19^{+}IgM^{+}$ (C and E) ASC populations. CAwMS (n = 8) and BALAwMS (n = 12); CAHD (n = 13) and BALAHD (n = 11). Error bars represent SD, p values determined by the 2-sided t test.

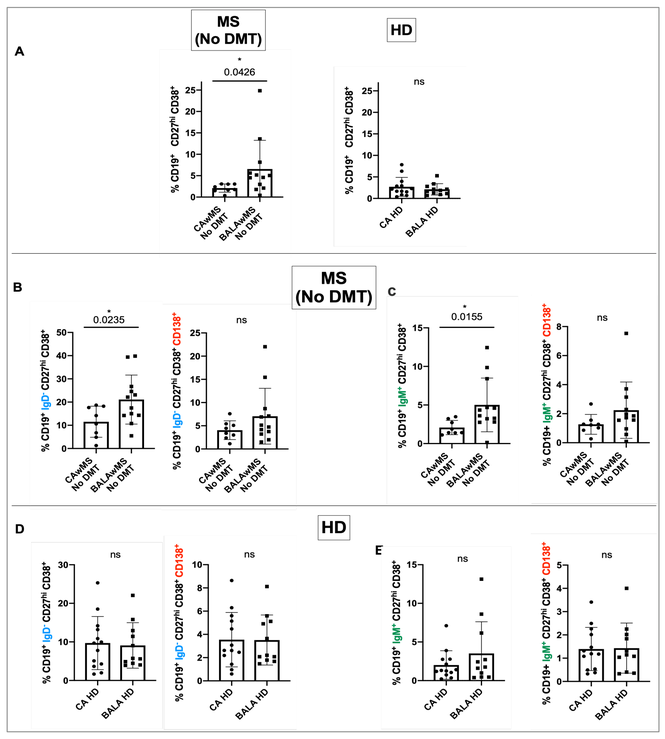
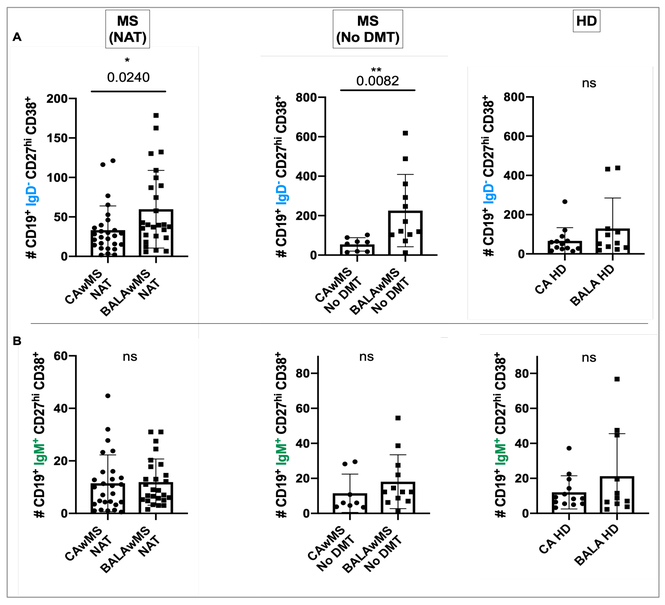


Figure 3. Class-switched ASCs are differentially enriched among subjects with MS of Black African or Latin American ethno-ancestral identity

Average number of circulating ASCs for each subject cohort as obtained through event counts derived from flow cytometry gates described prior. Class- switched $CD19^{+}IgD^{-}$ ASCs (A); unswitched $CD19^{+}IgM^{+}$ ASCs (B). Error bars represent SD, p values determined by the 2-sided t test.



Discussion

The major finding of our investigation was the significant ethnicity–associated differential of peripheral blood ASC subsets examined among participants with MS but not HD subjects. Both percent frequency as well as total event count were significantly elevated among BALAwMS relative to CAwMS suggesting differential underlying biology. Notably, only class-switched ASCs-, and not IgM^{+} ASC event counts were significantly elevated, implicating differential class-switch recombination as well as differentiation processes. ASC metrics were similar after adjusting for age, sex, and disease duration through multi-variate regression. Study limitations include singl—site convenience sample, cross-sectional design and use of ethnicity labels (which are ultimately societal constructs with vague biological association)[9]. Future work should incorporate multi-site longitudinal data, emphasize functional assays and employ genetic ancestry in conjunction with self-reported labels to more thoroughly clarify the relationship between ethnicity-associated heterogeneity and biological function.

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