Ancestry-based differences in the immune system are associated with lupus severity

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Abstract

Systemic lupus erythematosus (SLE) affects 1 in 537 of African American (AA) women, which is >2-fold more than European American (EA) women. AA patients also develop the disease at a younger age, have more severe symptoms, and a greater chance of early mortality. We used a multi-omics approach to uncover ancestry-specific immune alterations in SLE patients and healthy controls that may contribute to disease disparities. Cell composition, signaling, and epigenetics were evaluated by mass cytometry; droplet-based single cell transcriptomics and paired proteogenomics (scRNA-Seq/scCITE-Seq). Soluble mediator levels were measured in plasma and stimulated whole blood. Toll-like receptor (TLRs) pathways are activated by vaccination and microbial infection, and are also key drivers of autoimmune disease. We observed enhanced TLR3/4/7/8/9-related gene expression in immune cells from AA versus EA SLE patients. TLR7/8/9 and IFN α phospho-signaling responses were heightened even in immune cells from healthy AA versus EA controls. TLR stimulation of healthy AA and EA immune cells recapitulated the distinct ancestry-associated SLE immunophenotypes. Thus, healthy individuals show ancestry-based differences in innate immune pathways that could influence the course and severity of lupus and other diseases.

Introduction

Systemic lupus erythematosus (SLE) is a heterogeneous disease with varied environmental and genetic risk factors that may contribute to risks for development of the disease and to its disease course (1, 2). SLE prevalence is 2-3 times higher in African Americans (AA) than European Americans (EA) (3). AA ancestry is associated with greater disease activity, severity, and worse outcomes in SLE with a higher prevalence of end-stage renal failure and earlier mortality (2). Lupus remains a top 10 medical cause of death in AA women between 15 and 45 years of age (4), and individuals of African ancestry have a more abrupt and earlier age of SLE onset compared to those of EA descent (5).

Toll-like receptors (TLRs) 7 and 9 are pattern recognition receptors that sense single-stranded RNA and DNA, respectively, and are critical for type I IFN production and the generation of anti-viral immune responses. Activation of TLR7 and TLR9 by recognition of self nucleic acids is implicated in the pathogenesis and development of autoimmune disease (6). In lupus mouse models, TLR7 is a critical driver of disease, including nephritis, and anti-RNA antibodies (7), and TLR9 is important for the development of anti-DNA antibodies (7–9). Human lupus is associated with increased expression and activation of TLR7 and TLR9 on peripheral blood mononuclear cells, and activation of these TLRs induces IFN α production (10–13). Certain pathways driving lupus pathogenesis are known to be enhanced in AA versus EA populations, with greater frequencies of patients with elevated IFN α , B cell activation markers (CD86), and double negative 2 (DN2) autoantibody producing B cells (14–16). Patient ancestry has been shown to influence gene expression signatures which help define the molecular heterogeneity of SLE (17), with B cell-related expression signatures more heavily perturbed in AA versus EA, and greater plasma cell signatures found in AA SLE patients (17).

Differences in immune cell frequencies of healthy AA versus EA individuals have also been described, such as benign neutropenia, a greater CD8/CD4 ratio, and higher lymphocyte numbers, primarily B cells (18, 19). B cell numbers also differ by sex, with the highest cells/mL reported in AA females (20). Additionally, macrophages from AA individuals display a stronger inflammatory response to pathogens such as *Listeria*, *Salmonella*, and Influenza when compared to macrophages from EA individuals (21, 22).

Given ancestry-related differences in the immune response of healthy individuals, the current study investigated ancestry influences on immune response pathways linked to lupus, including TLR7, TLR9, and IFN α using a multi-omic, systems level approach. Ancestry-associated differences in dominant TLR signaling pathways that may influence lupus disease phenotype and severity were found in both healthy controls and SLE patients, suggesting the likelihood of inherited autoimmune-permissive immunologic patterns.

Results

Cohorts of AA and EA populations for immune analysis

To examine relationships between immune phenotypes, ancestry, and disease activity, the following were evaluated: 1) healthy controls (n=10 EA, n=8 AA), 2) SLE patients with inactive disease (SLE INACT: SLEDAI<4, n=10 EA, n=10 AA), and 3) SLE patients with active disease (SLE ACT: SLEDAI $\geq$ 4) (n=9 EA, n=11 AA) (Fig. 1 and Fig. S1). Patients were matched by self-reported ancestry, SLEDAI, sex, and medication use (Table S1). AA SLE ACT patients tended to be younger in age and there were no observable differences by ancestry in SLE INACT patients.

Clinical criteria were similar across SLE INACT patients of both ancestries. SLE-ACT patients displayed ancestry associated differences in the proportion with DNA binding autoantibodies (AA 60% vs EA 33%), leukopenia (AA 30% and EA 0%), and lymphopenia (AA 50% vs EA 33%) (Table S2). Autoantibody specificities differed slightly between AA and EA patients with AA patients having higher frequencies of RNP (26.3% EA vs 52.4% AA) or Sm/RNP (31.6% EA vs 61.9% AA) specific antibodies, and EA patients having higher frequencies of Ro (52.6% EA vs 23.8% AA) and La (15.8% EA vs 4.8% AA) specific antibodies. The number of specificities was higher in SLE ACT versus SLE INACT patients of both ancestries (Table S3-4, Fig. S2).

Lupus immune phenotypes vary by ancestry and disease activity

Mass cytometry was performed to investigate whole blood phenotypes and phospho-signaling (Fig. 1A). To assess changes in TLR cell signaling pathways, the mass cytometry panel was designed with 33 metal isotope-tagged monoclonal antibodies specific for cell lineage, phospho-proteins, activation, and homing markers that discern major immune cell subsets and subpopulations (Table S5). Single cell populations were visualized using a high-dimensionality reduction method (tSNE) that down-sampled the total cell population and unbiasedly clustered cells by likeness. Frequencies were determined by a

standard biaxial-gating scheme that incorporated all intact, live cells collected (Fig. 2A). The tSNE analysis included over 1.3 million cells (~17,000 cells/sample) and distinguished 22 phenotypically distinct clusters (Fig S3A). Marker expression of gated phenotype clusters is summarized for major lineage markers via tSNE plot (Fig. S3B) and by median intensity in a heatmap (Fig. S3C).

Concatenated files of all individuals in each disease group were used to create tSNE images, demonstrating that the immune cell composition of whole blood was different in all six cohorts, depending on both ancestry and disease scores (Fig. S3D). For instance, B cell and non-classical monocyte frequencies were greater in AA versus EA controls (Fig. 2A, Table S6-7). T cells, primarily CD8+ T cells, were also higher in SLE INACT AA versus EA patients. In contrast, neutrophil counts trended lower in AA versus EA controls, as expected (Fig. 2A) (18–20).

Increased disease activity in EA patients was associated with reduced frequencies of B cells, naïve and double negative (DN) (CD27-IgD-) B cells, CD4+ T cells, DN T cells, and basophils/eosinophils (Fig. 2A, Table S6-7). In AA samples, more severe disease was associated with reduced frequencies in non-classical monocytes, NK cells, CD8+ T cells, and CD141+ dendritic cells frequencies, as well as increased frequencies of memory B cells/plasmablasts (Fig. 2A, Table S6-7, Fig. S4). These data demonstrate that the immune compositions of whole blood from AA and EA populations differ in meaningful ways during SLE disease progression, and reflect some differences already present in healthy people.

Cell activation is higher in AA versus EA SLE patients

We next analyzed activated and inflammatory immune populations in the six cohorts. Briefly, we assessed the frequencies and mean metal intensity (MMI) in whole blood cell subsets by monitoring CD38, HLA-DR, CD11c, CD11b, and CD27 (Table S8-9). We found that CD38 antigen (Fig. 2B) and CD38+HLA-DR+ co-expressing activated T cells, monocytes, pDCs, and NK cells were elevated with increased disease activity in all SLE patients (Fig. S5A-H).

The T cell co-stimulatory molecule CD27 assists in antigen-specific expansion and supports survival of activated T cells (23). CD27, was more highly expressed in CD8+ T cells (Fig. 2C-D) and CD4+ T cells (Fig. 5I), across all AA versus EA samples. In addition, CD38 was more highly expressed in CD8+ T cells, DN T cells, and pDCs in AA versus EA SLE ACT patients (Fig. 2E-F, Fig. S5J).

Using markers in this panel, we did not observe changes in the frequencies of activated B cells between SLE ACT and SLE INACT disease activity (Table S8-9). However, AA versus EA naïve B cells expressed higher levels of IgD, even in the absence of disease (Fig. S5K-L). The immunoglobulin IgD is elevated in rheumatic disease, has a high proclivity toward binding to nuclear antigens, and induces potent inflammatory responses in myeloid cells (24, 25). In addition, the frequency of activated neutrophils increased with disease activity in AA patients, but not in EA patients (Fig. 2G-H).

To examine associations between cell populations and disease activity, linear regression analyses were performed with SLEDAI versus cell subset frequencies. In EA SLE patients, naïve B cell frequencies were

negatively associated with disease activity ($R^2=0.2014$) (Fig. S6A). In AA SLE patients, T cell frequencies (both CD8+ T cells ($R^2=0.2286$)) and CD4+ T cells ($R^2=0.2094$)) were negatively associated with disease activity, whereas activated CD38+ T cells were positively associated (Fig. S6B-D). In summary, CD8+ and DN T cell activation, pDC, and neutrophil activation were more prevalent in AA SLE ACT patients compared to EA SLE ACT patients. We infer that the activated and inflammatory immune populations in whole blood differ between AA and EA populations, both in healthy individuals and during SLE disease course.

H3K27ac in CD8+ T cells is higher with AA ancestry and disease severity

To investigate a potential relationship between immune cell epigenomes, ethnicity, and disease activity, we performed epigenetic landscape profiling using cytometry by time-of-flight (EpiTOF) (26, 27). The global levels of 40 chromatin modifications were measured on a single-cell level in 19 different cell populations (Fig. S7). Individuals were split into two biological replicates for testing (Fig. S8).

In most immune cell populations, diverse chromatin modifications were elevated in SLE patients compared to controls (Fig. 3A). However, pDCs, effector CD4+ and effector CD8+ T cells from SLE patients had lower levels of most chromatin modifications. SLE patients could be distinguished from healthy controls with high sensitivity (>85%) and specificity (>70%) using just three chromatin marks (H3K4me2, H3K4me3, and H4K20me1) (Fig. S9). These three chromatin marks had equal discriminatory capacity in both biological replicates.

We compared chromatin marks on 19 different immune cell populations between SLE patients and healthy controls using both AA and EA samples (Fig. 3B). The levels of H3K18ac (Fig. 3C) and H3K27ac (Fig. 3D) were higher in CD8+ T cells on all AA versus EA samples. Samples from AA patients could be distinguished from EA samples with high sensitivity and specificity (AUC=80%) using H3K27ac levels in CD8+ T cells alone (Fig. 3E). Further, H3K27ac in CD8+ T cells was higher in AA SLE ACT patients compared to SLE INACT patients, but this was not observed in EA patients (Fig. 3F). This was the only marker found to significantly distinguish disease activity (data not shown). These findings suggest that immune cell epigenomes of EA and AA populations are different, both in healthy individuals and during different SLE disease activity states.

IgG+ B cells are more frequent in AA SLE ACT patients

To investigate the transcriptional profiles of antigen-presenting cells in AA and EA SLE patients, we performed droplet-based scRNA-sequencing (10X Genomics) and protein genomics (CITE-seq) using CD2 depleted PBMCs on all study participants (Fig. 1C). After filtering out cells of low quality and batch normalization (Fig. S10-11), we obtained transcriptome datasets from ~90,000 cells, with over 30 million unique transcripts from PBMCs of all samples. Among these cells, 31,082 cells (35.3%) were from controls, 29,838 cells (33.9%) were from SLE INACT patients, and 27,133 cells (30.8%) were from SLE ACT patients (Fig. S12). Using graph-based clustering of uniform manifold approximation and projection

(UMAP), we captured the transcriptomes of 8 major cell types or subtypes according to the expression of canonical gene and protein markers (Fig. S13-14).

We used protein and transcriptome data to subdivide B cells into six subsets based on lineage markers (Fig. 4A-C). For both ancestries, increased disease activity was associated with a reduced frequency of naïve B cells (Fig. 4D). An increased frequency of age-associated B cells (ABCs), germinal center (GC) B cells, and plasmablasts (Fig. 4E-G), but not transitional or memory B cells (Fig. 4H-I), was also found. The reduced frequencies of naïve B cells in SLE ACT patients was also observed by mass cytometry, and coincided with increased gene expression of B cell differentiation, proliferation, and migration pathways, as well as decreased expression in apoptotic pathways (Fig. S15, Fig. 4J-K). IFN and RNA viral infection gene expression pathways were elevated in plasmablasts from patients with high disease activity (Fig. S16-17). Increased class-switching was also evident in lupus patients with higher disease activity (Fig. 4L-M). The frequency of IgG⁺ B cells was higher in SLE ACT versus SLE INACT in ABCs, GC B cells, plasmablasts, and memory B cells from AA, but not EA individuals (Fig. 4L-N, Fig. 18). EA individuals had greater frequencies of IgA⁺ plasmablasts in SLE ACT patients. These results suggest that naïve B cells may be more likely to differentiate and migrate during periods of heightened disease activity. This also supports a model in which increased disease severity of AA versus EA patients is associated with an increased frequency of cells producing high affinity IgG antibodies.

Myeloid transcriptomes define AA versus EA SLE disease activity

Monocytes clustered into 11 unique populations based on protein and gene expression markers (Fig. 5A-C). The frequency of intermediate monocytes was reduced in AA SLE ACT patients compared to controls, whereas the frequencies of IFN signature positive monocyte clusters were greater in SLE ACT EA patients (LYZ^{Hi}IFI6^{Hi}, ISG^{Hi}) and AA patients (ISG^{Hi}) (Fig. 5D-F). No significant differences were observed for all other monocyte populations (Fig. 5G, Fig. S19A-G).

Differences in IFN gene expression signatures in classical, non-classical, and intermediate cell subsets were assessed using predefined gene expression modules (28, 29). EA patients expressed similar elevated levels of IFN gene expression modules regardless of disease activity (Fig. 5H). In contrast, IFN pathway involvement in monocytes increased in AA SLE patients with active disease, particularly in intermediate monocytes (Fig. 5H). Apoptosis pathway involvement was reduced in intermediate monocytes from SLE ACT patients (Fig. S19H). These data are consistent with the hypothesis that expanded ISG^{Hi} monocyte populations contribute to heightened disease activity in both EA and AA patients, while greater IFN activation in intermediate monocytes in AA SLE ACT patients may accentuate activation and increased migration into affected tissues.

Transcriptionally defined dendritic cells could be split into three distinct subsets of CLEC9a⁺ conventional DCs (cDCs), CD1c⁺ cDCs, and pDCs (Fig. 5I-K, Fig. S19I-J). CD1c⁺ cDCs were elevated with disease severity in EA subsets, whereas pDCs were higher in all AA versus EA subsets. HLA Class I and II antigen

presentation transcripts were differentially expressed in cDCs of SLE patients (Fig. 5L). AA SLE ACT patients had the highest levels of HLA Class I expression compared to all other groups (Fig. 5L), whereas, EA SLE patients trended higher in expression levels of multiple HLA Class II transcripts. These data reveal transcriptome differences between dendritic cells of AA and EA individuals, both in health and during SLE disease progression.

TLR7/9 and IFN α signaling pathways are elevated in AA immune cells

Given that Toll-like receptors (TLRs) 7 and 9 and Type I Interferon are key drivers of murine autoimmune disease, we investigated whether differences in TLR and IFN signaling pathways underlie the observed ethnic differences in immune phenotypes, in health and SLE. To assess differences in TLR activity, Ingenuity Pathways Analysis (IPA) was first used to evaluate differentially expressed genes in monocyte and B cell clusters identified by UMAP of scRNA-sequencing (Fig. 6A-B, Table S10). TLR gene expression pathways were elevated in naïve B cells, memory B cell, and ABCs from SLE ACT AA patients versus EA patients (Fig. 6A). TLR gene expression pathways were also elevated in monocytes from AA versus EA SLE ACT patients, most notably among more activated/inflammatory monocyte populations: intermediate, CXCL8^{Hi}, polyfunctional, and CD14^{Low}CD16^{low} monocytes (Fig. 6B). The TLR4 signaling pathway (LPS) showed the greatest elevation, as previously reported (21), but TLR3, TLR7/8, and TLR9 pathways were all elevated in AA versus EA SLE ACT patients.

Next, 9 signaling proteins and 8 activation markers were monitored to assess TLR signaling. Phospho-CyTOF was performed before and after a 4 or 15 minute stimulation of whole blood (Fig. 6C, Table S11-18, Fig. S20-28), using cell surface markers to discern 8 major cell lineages (Fig. S20). IFN α mediated induction of pSTAT5 was diminished in most cell lineages from AA patients versus AA healthy controls (Fig. S29-30). TLR7/8 and TLR9 induced phospho-signaling markers and activation proteins were also lower in multiple cell lineages from AA SLE patients compared to AA healthy controls (Fig. S29-33). Lower fold changes were not a result of SLE patients cells being maximally stimulated, as they had lower total levels of phospho-signaling proteins following stimulation (Fig. S30B). TLR7/8- and TLR9- mediated induction of p-p38 and pERK1/2 were also lower in EA patients versus healthy controls, which may suggest exhaustion of these pathways from persistent stimulation in patients.

There were significant differences in TLR signaling responses between AA and EA healthy controls. For instance, relative to EA, AA samples showed increased IFN α -mediated induction of pSTAT5 as well as increased PMA/Ionomycin-, TLR7/8- and TLR9-mediated induction of cCASP3, pCREB, pERK1/2, pPLCy2, Syk, and p-STATs (Fig. 6C). The TLR9 responses of B cells from healthy AA versus healthy EA were particularly more prone to activation, with higher expression of CD38, CD27, HLA-DR, CD11c and CD11b after stimulation. These data suggest that TLR7/8/9 and IFN α signaling pathways are inherently different in healthy AA versus EA individuals, which may influence lupus disease manifestations and severity.

TLR stimulation of healthy AA and EA immune cells recapitulates ancestry-associated SLE immunophenotypes

AA SLE ACT versus SLE INACT patients have fewer NK cells, T cells, non-classical monocytes, and more memory B cells/plasmablasts (Fig. 2A), whereas EA SLE ACT versus SLE INACT patients have fewer B cells (Fig. 2A). We hypothesized that the observed ancestry-dependent differences in TLR7, TLR9, and IFN signaling in healthy controls might contribute to the differences in the immune cell frequencies of SLE patients.

To test this hypothesis, PBMCs were obtained from 10 AA and 10 EA healthy controls (matched by age, sex, and BMI), and cells were stimulated *in vitro* with TLR7/8 agonist, TLR9 agonist, or IFN α alone or in combination, for 7 days. All controls were female, had a mean age of 29.2 years, and no clinical symptoms of autoimmune disease as determined by a connective tissue screening questionnaire (CSQ) (30), were anti-nuclear antibody negative (ANA) by Hep2 immunofluorescence and by Bioplex 2200, and had no anti-cyclic citrullinated peptide (CCP) or rheumatoid factor (RF)-specific autoantibodies (data not shown).

The immune cell compositions following stimulation of PBMCs with TLR7/8 (R848) and/or TLR9 (CpG) agonists were significantly different for AA versus EA healthy controls (Fig. 7A-B). In particular, the combination of TLR7 and TLR9 agonists resulted in increased T cells (Fig. 7C) and NK cells (Fig. 7D) in AA versus EA healthy controls, but no differences in total B cells (Fig. 7E) and myeloid cells (Fig. 7F). B cells were then classified into 6 subsets using CD27 and IgM gating via biaxial plots (Fig. 7G). Simultaneous treatment with TLR7 and TLR9 agonists resulted in fewer naïve B cells and increased class-switched plasmablasts in AA versus EA healthy controls (Fig. 7H-M). AA samples produced more DN B cells in response to R848, R848+IFN α , and R848+CpG+IFN α (Fig. 7I), and more class-switched memory B cells and class-switched plasmablasts in response to R848+CpG (Fig. 7J-K). IgG levels were also higher in AA versus EA, but no differences were observed in IgA or IgM following TLR7/8, TLR9, or IFN α stimulation (Fig. 7L-M, Fig. S34). The response to simultaneous TLR7/8 and TLR9 stimulation in AA samples induced a stronger T cell, NK cell, and class-switched B cell response, whereas EA individuals had predominantly more naïve B cells. These changes recapitulate the cell population changes seen in AA and EA SLE patients.

Elevated pro-inflammatory cytokines in AA SLE patients and altered responses to TLR-driven cytokine pathways in lupus patients

Next, we tested whether increased disease activity and altered immune responses in EA and AA SLE patients were associated with alterations in chemokine and soluble mediator profiles. We assessed 39 different mediators by multiplex bead-based assays or ELISA (Fig. 1B) and found that plasma from AA

SLE patients had increased levels of pro-inflammatory cytokines and other inflammatory regulators when compared to plasma from EA SLE patients (Fig. 8A-G, Fig. S35A-G, Table S19-20). The chemokine, Eotaxin, which helps to recruit eosinophils, was the only cytokine that was significantly increased in EA versus AA patients (Fig. 8H). Stem cell factor (SCF or c-kit) and MCP-1 levels best correlated with disease activity (Fig. 8I-J). These data suggest that higher levels of pro-inflammatory cytokines contribute to altered and enhanced disease activity in AA SLE patients.

To determine whether dysregulated signaling pathways contribute to the differences in soluble mediators, we stimulated whole blood from controls, SLE INACT and SLE ACT patients with vehicle, R848/CpG/LPS (TLR), PHA, or PMA+Ionomycin for 24 hours, collected supernatants and measured the levels of 39 soluble mediators (Fig. 1A-B, Fig. S36). Cytokine levels were already significantly elevated in unstimulated culture supernatants of SLE patients compared to controls, most notably in AA SLE ACT patients (Fig. S35H-L). Following TLR stimulation of SLE ACT whole blood, a subset of pro-inflammatory cytokines was increased, including CXCL13, IL-13, IL-6, SCF, and TNF- β , with trends higher in most other cytokines (Fig. 8K, Fig. S37, Table S21-22). In contrast, TLR-mediated stimulation of Type I and Type II IFN cytokine pathways were specifically reduced in SLE patients versus healthy controls, particularly for SLE ACT patients (Fig. 8L-N). Diminished Type I IFN responsiveness was specific to TLR stimulation, whereas Type II IFN reduction were also observed following PHA and PMA-Ionomycin stimulation (Fig. S38-40, Table S23-26). The reduction in TLR-induced IFN α levels was associated with higher IFN gene signatures in classical and non-classical monocytes, suggesting that this may be due to exhaustion of the TLR-IFN cytokine pathway (Fig. 8O-P). The diminished IFN response was not significantly different between EA or AA patients; however, EA SLE ACT patients produced higher levels of the regulatory cytokines IL-1RA and IL-10, which may mitigate TLR-induced inflammation (Fig. S37B-C). The altered TLR response in lupus patients, with diminished early IFN production and increased pro-inflammatory cytokine levels, indicates external or internal stressors that trigger the TLR pathway may be associated with altered responses to infection or induction of disease flare. These alterations are more severe in patients with increased disease activity.

Discussion

In this study, we identify a significant impact of ancestry on immune composition and cellular signaling responses triggered by viral pathways. The likelihood that this shapes the immune phenotype in SLE is supported by the markedly stronger response of AA healthy controls to TLR7/8, TLR9, and IFN α stimulation pathways known to drive lupus pathogenesis. These results were consistent across multiple platforms with higher TLR gene expression pathways, greater cell activation and phospho-signaling profiles, and amplified *in vitro* responses seen in AA individuals. Past studies have demonstrated increased pro-inflammatory responses to bacterial pathogens and influenza in AA patients (21, 22), higher rates of inflammatory diseases (31), and greater lupus and COVID-19 disease severity (2, 32, 33). We speculate that stronger TLR7/8/9 responses contribute to greater disease burden, earlier age of onset, and worsened disease symptoms known to occur in AA SLE patients.

Ancestry-related differences in cell composition and the TLR7/8/9 and IFN α responses may be explained by a combination of genetic and environmental factors (22). Benign neutropenia, which helps account for lower frequencies of neutrophils and higher lymphocyte counts in the circulation of AA individuals, has been linked to rs2814778 (G) variant of the gene encoding atypical ACKR1, the duffy antigen receptor for cytokines (DARC) (34). It is possible that higher frequencies of TLR7 and TLR9 expressing cells in the circulation may contribute to greater expansion of these populations in response to stimuli in AA. Higher expression of TLR7 and TLR9 have been reported on AA versus EA SLE patient PBMCs (13). Women of AA descent were also found to carry significantly more allelic variants known to upregulate pro-inflammatory cytokines that may influence the strength of TLR7/8/9 responses (35). Environmental factors, such as exposure to Epstein-Barr virus and cytomegalovirus trigger the same TLR pathways, and may further prime immune responses to favor increased pro-inflammatory cytokine production. AA individuals also have a disproportionately higher prevalence of EBV, CMV, and HSV-1 antibodies that associate with socioeconomic variables (36).

One of the differences in the immune response of AA SLE ACT patients was increased plasmablast, memory B cell, ABC, and germinal center B cell involvement contributing more evidence to a stronger B cell phenotype in AA SLE patients (14–17, 37–39). However, we found that this phenotype is not unique to the SLE patients in the AA population. Healthy AA controls generated more IgG class-switched memory B cells and plasma cells in response to TLR7/8 and TLR9 stimuli. This expansion of IgG⁺ B cells, and the ability to class-switch to IgG, requires at least 3 cell divisions, the presence of T cell stimulation, and a proper cytokine milieu that includes IL-13, IL-4, and/or IL-10 (40). We found IL-13 and IL-4 in higher concentrations in the plasma of AA SLE patients with greater T cell activation and CD38 and CD27 co-stimulatory marker expression in AA SLE patients.

In addition to an altered B cell axis in AA versus EA SLE patients, a more extensive AA-associated SLE phenotype was found that included greater CD8⁺ T cell, NK cell, non-classical monocyte, and DC involvement. We previously found T cells to be increased in SLE patients, with greater numbers in AA SLE patients, and increased T cell activation profiles in AA patients prior to lupus development (41).

In this study, simultaneous treatment with TLR7/8 and TLR9 agonists drove increased T cells in AA healthy control cultures. Effective activation of naïve T cells requires three distinct signals: recognition of peptide-HLA complex on APCs, co-stimulatory molecule interaction of the T cell and APC, and pro-inflammatory cytokine signals from the external environment. Dendritic cells in AA SLE ACT patients express more β -2 microglobulin (B2M) and other HLA class I molecules, including HLA-A, HLA-B, and HLA-C, compared to EA SLE ACT patients and AA controls and SLE INACT patients that may increase HLA class I self-peptide presentation. The T cells of AA samples exhibited higher expression of CD27, a co-stimulatory marker that supports antigen-specific expansion and sustains activation. Further, elevated levels of cytokines TNF α , IL-4, IL-13, SCF and others in AA patients can increase T cell differentiation and expansion. A skewing of the T cell response with decreases in CD8⁺ T cells in AA SLE ACT versus CD4⁺ T cells in EA patients was also observed. Decreases in effector cell populations with disease onset have been previously seen in autoimmune disease as activated cells migrate to affected tissues (42–44).

Heightened gene expression of trans migratory transcripts, increased activation markers, and decreased apoptosis transcripts in these cell populations suggest cells become activated and leave the periphery.

T cells were correlated with SLE disease activity in AA patients. CD8⁺ T cell epigenetic modifications in H3K18ac and H3K27ac, both associated with higher activation of transcription, was enhanced in AA SLE ACT patients. Our previous work has shown, that H3K27ac is correlated with cytokine production (45), namely TNF α , which this study found was also elevated in AA versus EA SLE patients. We found that treatment with the histone acetyl transferase inhibitor A-485 led to a concentration-dependent decrease in global histone H3K27ac levels that did not affect cell viability (45). More importantly, treatment with A-485 led to a major diminution in the frequency of IL-1 β and TNF α suggesting this may be an effective target in AA SLE patients.

CD8⁺ T cells are also the predominant infiltrating immune cell subset in SLE patients with class III or IV nephritis, which is more prevalent in AA patients (46). Further, single cell transcriptional analyses revealed NK cells and ABCs to significantly contribute to immune infiltrates in the kidney (47). Accumulation of CD8⁺ T cells in periglomerular areas was associated with worse outcome and greater disease severity (46, 48). Heightened expression of HLA Class I in DCs and increased frequencies of inflammatory non-classical/intermediate monocytes in AA versus EA SLE ACT patients may direct the immune response towards more auto-aggressive CD8⁺ T cells due to increased cross-presentation (49, 50). Higher SCF levels in the plasma may also contribute to increased dendritic cell activation and T cell differentiation (51). Stronger TLR7/8/9 and IFN α signaling that drive heightened CD8⁺ T cell, NK cell, monocyte/DC, and B cell responses likely contribute to a higher risk for lupus nephritis in AA patients.

While heightened TLR gene expression pathways were found in SLE patients, phospho-signaling responses following whole blood TLR7/8 and TLR9 stimulation were reduced. This is consistent with previous reports that correlated diminished responses to transcriptional signatures of cellular senescence, stress, and shortened telomere lengths (52). In the current study this evidence of exhaustion is enhanced in AA versus EA patients with heightened disease activity. Hastened telomere shortening is known to contribute to earlier aging and mortality in SLE patients (53). Stronger TLR7/8/9 signaling pathways that drive exhausted responses, stress, and telomere shortening may lead to earlier mortality in AA SLE patients.

Lupus patients, specifically those with heightened disease activity and lupus nephritis, are known to be particularly prone to infections, which are a leading cause of morbidity and mortality in SLE (54–56). While part of the high infection risk is likely due to immunosuppressive medications, alterations in the immune response due to lupus may also contribute to heightened risk. Following stimulation with TLR agonists, lupus patients with high disease activity produce significantly less Type I and II IFN and IFN-associated cytokines regardless of ancestry. This decreased IFN response is directly associated with higher IFN gene signature scores in monocytes at baseline. Although IFN levels were reduced compared to controls, other pro-inflammatory cytokine levels remained high, a phenotype consistent with aberrant and severe immune responses in certain viral infections (57, 58). EA SLE ACT patients were distinguished

from AA SLE ACT patients by higher levels of the regulatory cytokines IL-10 and IL-1RA that may help dampen pro-inflammatory inflammation in the context of a dysregulated immune response. Exhausted IFN pathways from chronic inflammation in lupus patients may contribute to greater infection risk, more severe manifestations, and lead to the lupus flares that are more common in AA patients.

TLR7 and TLR9 pathway activation is also required for effective responses to vaccines and viral infections, such as COVID-19, which disproportionately affects AA and Hispanics more severely than EA. Severe COVID-19 infection is characterized by a stronger CD8+ T cell response, whereas mild COVID-19 patients have reduced frequencies and a more exhausted CD8+ T cell phenotype (59). Further, severe COVID-19 patients have an increased and more highly activated NK cell response (60). Multi-system inflammatory syndrome in children (MIS-C), which occurs in some individuals after COVID-19 infection, is also more prevalent in AA and Hispanic children and is defined by a lack of exhausted CD8+ T cells that maintain the inflammatory environment (61). AA individuals are reported to have higher antibody titers to rubella, measles, pertussis, and influenza vaccinations (62–65). These phenotypes are consistent with the heightened response to TLR7/8 and TLR9 pathways observed in healthy AA controls, and are the same immune subsets altered during heightened SLE disease in AA patients. While these responses may be helpful in vaccination and promoting an effective memory response, it may predispose individuals of AA descent to more severe disease manifestations during infection and autoimmune disease.

Limitations of the current study include the small size and the cross-sectional study design, which allows capture of the immune profile at only one timepoint. A secondary cohort with a larger sample size to confirm the findings of this study including a longitudinal assessment of AA versus EA SLE patients with changes in disease activity over time will be important to test the robustness of these findings. Despite the small sample size, the consistency of phenotype differences across multi-omic platforms helps substantiate the ancestry-based results that have come to light in this study, which may be critical clues in developing precision medicine for SLE. However, it is also important to acknowledge that ancestry alone is an imperfect way to approach individual phenotyping and therefore it is critical to avoid pigeonholing best medical practice in the future based only on racial background.

The results of this project provide new insight into the contribution of ancestry in driving immune composition and responses in SLE disease activity. Heightened TLR7/8, TLR9 and IFN α signaling pathways in the AA immune response support immune phenotypes seen in response to infection and vaccines, and in autoimmune disease. Increased CD8+ T cell, NK cell, B cell, monocyte, and DC activity in AA individuals after stimulation of TLR7/8/9 may contribute to increased disease severity and early mortality in AA SLE patients, and these pathways may be targeted for more personalized treatments. Our results stress the importance of ancestry assessment in the illumination of immune phenotypes that may be key to outcomes across medical fields.

Materials And Methods

Study Population and Sample Collection. All experiments using human samples were conducted in accordance with the Helsinki Declaration and approved by the Institutional Review Board at the Oklahoma Medical Research Foundation (OMRF). Blood was collected at OMRF from 18 healthy controls (10 EA and 8 AA) and 40 lupus patients (19 EA and 21 AA) following written consent and based on self-reported ancestry. Clinical history and demographic data were recorded at the time of collection. Lupus patients were divided by disease activity into either high (SLEDAI ≥ 4) or low disease activity (SLEDAI < 4). Individuals were matched across 6 ancestry/disease groups (EA Control, EA SLE INACT, EA SLE ACT, AA Control, AA SLE INACT, AA SLE ACT) by gender, age ± 5 years, and medication use. AA SLE and EA SLE patients were also matched by disease activity using SELENA-SLEDAI (66). SLE patients fulfilled the American College of Rheumatology (ACR) and SLICC criteria for SLE classification (67). Whole blood was stimulated (see below), stabilized and samples were stored in liquid nitrogen and shipped frozen to the Human Immune Monitoring Center (HIMC) at Stanford University for mass cytometry analyses. PBMCs were isolated using Lymphocyte Separation Medium (Mediatech, Inc.) and stored in barcoded vials with freezing media (20% human serum/10% DMSO in RPMI). Plasma samples were collected and stored at -80°C until use.

Autoantibody Screening. SLE patients and healthy controls were assessed for ANA positivity and specificity via indirect immunofluorescence (IIF) and for 11 serum autoantibody specificities using the Bioplex 2200® system (Bio-Rad Technologies). IIF testing was performed by CAP-CLIA certified (Morris Reichlin, MD) Clinical Immunology Laboratory using the NOVA Lite IIF (Inova Diagnostics, Inc.) following the manufacturer's recommended protocols and cutoffs (68–70). Briefly, Bioplex 2200 ANA tested autoantibody specificities include dsDNA, chromatin, Ro/SSA, La/SSB, Sm, SmRNP, RNP, Centromere B, Ribosomal P, Scl-70, and Jo-1. All autoantibodies, except anti-dsDNA, were reported in antibody index (AI) units based on a fluorescent intensity range of 0-8. The manufacturer-specified cutoff was used to determine positivity (positive ≥ 1 AI) for all auto-specificities, except for anti-dsDNA where semi-quantitative values were reported as IU/mL with positive ≥ 10 IU/mL.

Whole Blood Stimulations. Heparinized whole blood was collected and rested for 1 hour at 37°C prior. Whole blood (200 μL) was added to cluster tubes containing 50 μL of stimuli, either media, LPS (Final Conc: 1 $\mu\text{g}/\text{mL}$), R848 (10 μM), CpG (10 $\mu\text{g}/\text{mL}$), PMA and Ionomycin (10 ng/mL (PMA), 1 $\mu\text{g}/\text{mL}$ (Iono)), or IFN α (10,000 U/ mL) for 4 minutes (TLR stimulations) or 15 minutes (IFN α , PMA/Ionomycin). Cells were immediately stabilized with a proteomic stabilizer (SmartTube, Inc) following manufacturer's instructions and store at -80°C until Phospho-CYTOF staining and sample acquisition. Stimuli were purchased from either Sigma, (PHA, PMA, LPS, and Ionomycin), PBL Assay Source, or InvivoGen (CpG ODN 2006 and Resiquimod (R848)). A second aliquot of whole blood was diluted 1:2 (400 μL total) with complete RPMI (RPMI supplemented with 10% fetal calf serum, penicillin streptomycin, and glutamine) in cluster tubes. Either 100 μL media (unstimulated), PHA-Ionomycin (1 $\mu\text{g}/\text{mL}$ each), LPS/R848/CpG (1 $\mu\text{g}/\text{mL}$, 10 μM , and 10 $\mu\text{g}/\text{mL}$), or PMA-Ionomycin (10 ng/mL , 1 $\mu\text{g}/\text{mL}$) were added to cluster tubes. Cells were incubated overnight (24 hours) after which cells were pelleted and supernatants collected for soluble mediator assessment.

Phospho-signaling Mass Cytometry Staining and Acquisition. Phospho-CyTOF was performed at the Human Immune Monitoring Center at Stanford University. Upon thawing, samples were washed and surface stained for 30 min at room temperature (Table S5). Cells were washed twice, permeabilized with 100% methanol and kept at -80°C overnight. The next day, cells were washed and resuspended in intracellular antibody cocktail for 30 min before washing twice. Cells were resuspended in 100 mL iridium-containing DNA intercalator and incubated for 20 minutes. Cells were washed once with cyFACS buffer and twice with MilliQ water. Cells were diluted to 750×10^5 cells/mL in MilliQ water and acquired on CyTOF. Data analysis was performed using viSNE in Cytobank to determine cell populations, and fold change of the 95th percentile was calculated for all 9 signaling molecules and 8 major cell populations.

Epigenetic Landscape Profiling Using Mass Cytometry (EpiTOF). As part of the ACE Network Collaborative Project, EpiTOF was performed as previously described (27), on 53 out of the 58 individuals in this study (10 EA Control, 10 SLE INACT, 8 SLE ACT, 8 AA Control, 9 AA SLE INACT, 8 SLE ACT). AA and EA samples were stained with an EpiTOF antibody panel containing 15 surface markers, 40 chromatin modification markers, and 2 histone controls (Fig. S7). Briefly, PBMCs were thawed and incubated in RPMI 1640 media (ThermoFischer) with 10% FBS (ATCC) for 1 hour prior to processing. Cisplatin (10 μ M) (ENZO Life Sciences) was added for viability staining for 5 minutes before quenching with CyTOF Buffer. Cells were washed and stained with 15 immunophenotypic markers. Following extracellular marker staining, cells were washed 3 times and fixed in 1.6% PFA (Electron Microscopy Sciences) for 15 minutes at RT. Cells were permeabilized with 1 mL ice-cold methanol (Fisher Scientific) for 20 minutes at 4°C. CyTOF buffer was added to stop permeabilization followed by 2 PBS washes. Mass-tag sample barcoding was performed following the manufacturer's protocol (Fluidigm). Individual samples were then combined and stained with 42 intracellular antibodies in CyTOF buffer containing Fc receptor blocker (BioLegend) overnight at 4°C. The following day, cells were washed twice and stained with 191/193Irr DNA intercalator (Fluidigm) in PBS with 1.6% PFA for 30 minutes at RT. Cells were washed, filtered, and resuspended with calibration beads and analyzed on CyTOF2 (Fluidigm) in Stanford Shared FACS Facility. 200,000 cells were collected for each subject in the study. Individuals were split into two biological replicates with matched demographics that were distributed evenly between AA and EA individuals.

In Vitro TLR7, TLR9, and IFN α Stimulations with Healthy Controls. PBMCs from 20 healthy controls were recruited through health fairs, as previously described (41). 10 EA and 10 AA healthy individuals were selected for *in vitro* stimulation studies. To ensure no individuals had any autoimmune disease manifestations, only controls that were ANA negative by both IIF and Bioplex, negative for CCP and RF antibodies, and had scored 0 on CSQ in all autoimmune disease categories were selected. Individuals were all female and were matched across ancestries by age and BMI. Cells were plated at 1×10^6 cells/mL (900 μ l) in a 24 well plate, where 100 μ l of stimuli was added in the following stimulation conditions: media (unstimulated), R848, CpG, IFN α , R848+IFN α , CpG+IFN α , R848+CpG, R848+CpG+IFN α . R848 and CpG were both used at a final concentration of 300 ng/mL, while IFN α was used at 1000 U/mL. Cells were incubated with stimuli at 37°C, 5% CO $_2$ for 7 days. Plates were spun down, supernatants were

collected and stored at -80°C until use for antibody ELISAs. Cells were collected for B cell and other cell subset assessment using an 8 marker antibody panel for flow cytometry. Cells were washed, incubated in Fc block (BD Biosciences) and stained with CD3 (UCHT1), CD19 (HIB-19), CD27(L128), CD38 (HIT2), IgD (I-A62), CD11c (B-Ly6), IgM (G20-127), and CD24 (ML5) from BD Biosciences and BioLegend at the manufacturer's recommended concentrations. Cells were washed and immediately run on an LSRII (BD Biosciences).

Total Antibody ELISAs. IgM, IgA, and total IgG ELISAs (ThermoFisher/Invitrogen) were run on culture supernatants following 7 day PBMC incubation for unstimulated and stimulated samples. Samples were thawed after storage at -80°C and diluted. The assays were performed according to the manufacturer's instructions. A dilution of 1:50 for IgM, 1:40 for IgG, and 1:20 for IgA was used for calculations to determine µg or ng/mL.

Soluble Mediator Measurements. Plasma levels of CXCL13, Eotaxin, GM-CSF, GRO-α, IFNα, IFNγ, IL-1α, IL-1β, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-2, IL-21, IL-22, IL-23, IL-27, IL-31, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, sCD40L, SCF, SDF-1α, sICAM-1, TNF-β, TNF-α, and IL-1RA were assessed by xMAP multiplex assay (eBioscience/Affymetrix). Data were obtained using a Bio-Rad Bioplex 200 system (Bio-Rad Technologies). Further, whole blood culture supernatants were also assessed using XMAP multiplex assays. Plasma levels and whole blood culture supernatants of BLyS were assessed using enzyme linked immunosorbent assay (ELISA) per manufacturer's protocol (R&D Systems).

Statistics. tSNE analysis were performed using Cytobank Premium SAAS (software as a service) on the Amazon Web serviced cloud (71). FCS files were uploaded to Cytobank and gated off live intact singlet cells. Concatenated files of 430,000 cells were used for representative tSNE images and cell subset profiling. Frequencies of cell subsets were exported from tSNE for cell number calculations and analysis. Traditional bivariate gating was performed in Cytobank. Cytokine data were non-normally distributed; therefore, continuous data were analyzed using the Kruskal-Wallis test in TIBCO Spotfire (version 11.4.0 LTS) (TIBCO Software, Inc., Boston, MA) with Wilcoxon-Mann-Whitney two-tailed test for two-group comparisons. The *q* values were calculated using the GraphPad PRISM (version 9.2.0) for Windows (GraphPad Software, San Diego, CA) two-stage step-up method of Benjamini, Krieger, and Yekutieli to correct for multiple comparisons and estimate the false discovery rate to control for the expected proportion of incorrectly rejected null hypotheses. All dot plots were generating using GraphPad PRISM or R package and heatmaps were generated using TIBCO Spotfire.

Declarations

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Competing interests: OMRF has licensed intellectual property of JAJ to Progentec Biosciences. Otherwise, the authors declare no competing interests.

Data and materials availability: All RNA-sequencing data that support the findings of this study have been deposited in GEO, accession number GEO189050. All other data (CyTOF, EpiTOF, and cytokine-MBAA) are being uploaded to IMMPort and will be available prior to publication under the study title *Molecular Heterogeneity of SLE Disease Activity* and study ID SDY1879.

References

1. I. Gergianaki, A. Bortoluzzi, G. Bertsias, Update on the epidemiology, risk factors, and disease outcomes of systemic lupus erythematosus. *Best Pract Res Cl Rh* **32**, 188–205 (2018).
2. M. J. Lewis, A. S. Jawad, The effect of ethnicity and genetic ancestry on the epidemiology, clinical features and outcome of systemic lupus erythematosus. *Rheumatology* **56**, 67–77 (2017).
3. G. Stojan, M. Petri, Epidemiology of systemic lupus erythematosus: an update. *Curr Opin Rheumatol* **30**, 144–150 (2018).
4. E. Y. Yen, R. R. Singh, Brief Report: Lupus-An Unrecognized Leading Cause of Death in Young Females: A Population-Based Study Using Nationwide Death Certificates, 2000-2015. *Arthritis Rheumatol* **70**, 1251–1255 (2018).
5. G. S. Alarcon *et al.*, Systemic lupus erythematosus in three ethnic groups: II. Features predictive of disease activity early in its course. LUMINA Study Group. Lupus in minority populations, nature versus nurture. *Arthritis Rheum* **41**, 1173–1180 (1998).
6. S. Fillatreau, B. Manfroi, T. Dorner, Toll-like receptor signalling in B cells during systemic lupus erythematosus. *Nat Rev Rheumatol* **17**, 98–108 (2021).
7. S. R. Christensen *et al.*, Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. *Immunity* **25**, 417–428 (2006).
8. L. Bossaller *et al.*, TLR9 Deficiency Leads to Accelerated Renal Disease and Myeloid Lineage Abnormalities in Pristane-Induced Murine Lupus. *J Immunol* **197**, 1044–1053 (2016).
9. K. M. Nickerson, Y. Wang, S. Bastacky, M. J. Shlomchik, Toll-like receptor 9 suppresses lupus disease in Fas-sufficient MRL Mice. *PLoS One* **12**, e0173471 (2017).
10. S. Mortezaagholi *et al.*, Evaluation of TLR9 expression on PBMCs and CpG ODN-TLR9 ligation on IFN- α production in SLE patients. *Immunopharmacol Immunotoxicol* **39**, 11–18 (2017).
11. K. Sakata *et al.*, Up-Regulation of TLR7-Mediated IFN- α Production by Plasmacytoid Dendritic Cells in Patients With Systemic Lupus Erythematosus. *Front Immunol* **9**, 1957 (2018).

12. A. Komatsuda *et al.*, Up-regulated expression of Toll-like receptors mRNAs in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Clin Exp Immunol* **152**, 482–487 (2008).
13. B. D. Lyn-Cook *et al.*, Increased expression of Toll-like receptors (TLRs) 7 and 9 and other cytokines in systemic lupus erythematosus (SLE) patients: ethnic differences and potential new targets for therapeutic drugs. *Mol Immunol* **61**, 38–43 (2014).
14. L. C. Menard *et al.*, B cells from African American lupus patients exhibit an activated phenotype. *JCI Insight* **1**, e87310 (2016).
15. S. A. Jenks *et al.*, Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. *Immunity* **49**, 725-739 e726 (2018).
16. C. E. Weckerle *et al.*, Network analysis of associations between serum interferon-alpha activity, autoantibodies, and clinical features in systemic lupus erythematosus. *Arthritis Rheum* **63**, 1044–1053 (2011).
17. M. D. Catalina *et al.*, Patient ancestry significantly contributes to molecular heterogeneity of systemic lupus erythematosus. *JCI Insight* **5**, (2020).
18. D. S. Freedman *et al.*, Black/white differences in leukocyte subpopulations in men. *Int J Epidemiol* **26**, 757–764 (1997).
19. E. Beutler, C. West, Hematologic differences between African-Americans and whites: the roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume. *Blood* **106**, 740–745 (2005).
20. D. J. Tollerud *et al.*, The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers. *J Clin Immunol* **9**, 214–222 (1989).
21. Y. Nedelec *et al.*, Genetic Ancestry and Natural Selection Drive Population Differences in Immune Responses to Pathogens. *Cell* **167**, 657-669 e621 (2016).
22. H. E. Randolph *et al.*, Genetic ancestry effects on the response to viral infection are pervasive but cell type specific. *Science* **374**, 1127–1133 (2021).
23. J. Hendriks *et al.*, CD27 is required for generation and long-term maintenance of T cell immunity. *Nat Immunol* **1**, 433–440 (2000).
24. K. Chen, A. Cerutti, New insights into the enigma of immunoglobulin D. *Immunol Rev* **237**, 160–179 (2010).
25. C. Gutzeit, K. Chen, A. Cerutti, The enigmatic function of IgD: some answers at last. *Eur J Immunol* **48**, 1101–1113 (2018).
26. P. Cheung *et al.*, Single-cell epigenetics - Chromatin modification atlas unveiled by mass cytometry. *Clin Immunol* **196**, 40–48 (2018).
27. P. Cheung *et al.*, Single-Cell Chromatin Modification Profiling Reveals Increased Epigenetic Variations with Aging. *Cell* **173**, 1385-1397 e1314 (2018).

28. R. Banchereau *et al.*, Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell* **165**, 1548–1550 (2016).
29. D. Chaussabel, N. Baldwin, Democratizing systems immunology with modular transcriptional repertoire analyses. *Nat Rev Immunol* **14**, 271–280 (2014).
30. E. W. Karlson *et al.*, A connective tissue disease screening questionnaire for population studies. *Ann Epidemiol* **5**, 297–302 (1995).
31. R. Pennington, C. Gatenbee, B. Kennedy, H. Harpending, G. Cochran, Group differences in proneness to inflammation. *Infect Genet Evol* **9**, 1371–1380 (2009).
32. M. Doumas *et al.*, COVID19 and increased mortality in African Americans: socioeconomic differences or does the renin angiotensin system also contribute? *J Hum Hypertens* **34**, 764–767 (2020).
33. C. W. Yancy, COVID-19 and African Americans. *JAMA* **323**, 1891–1892 (2020).
34. J. Duchene *et al.*, Atypical chemokine receptor 1 on nucleated erythroid cells regulates hematopoiesis. *Nat Immunol* **18**, 753–761 (2017).
35. R. B. Ness, C. L. Haggerty, G. Harger, R. Ferrell, Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *Am J Epidemiol* **160**, 1033–1038 (2004).
36. A. S. Delaney, W. Thomas, H. H. Balfour, Jr., Coprevalence of Epstein-Barr Virus, Cytomegalovirus, and Herpes Simplex Virus Type-1 Antibodies Among United States Children and Factors Associated With Their Acquisition. *J Pediatric Infect Dis Soc* **4**, 323–329 (2015).
37. L. L. Ritterhouse *et al.*, B lymphocyte stimulator levels in systemic lupus erythematosus: higher circulating levels in African American patients and increased production after influenza vaccination in patients with low baseline levels. *Arthritis Rheum* **63**, 3931–3941 (2011).
38. T. Iwamoto, J. Dorschner, M. Jolly, X. Huang, T. B. Niewold, Associations between type I interferon and antiphospholipid antibody status differ between ancestral backgrounds. *Lupus Sci Med* **5**, e000246 (2018).
39. S. R. Crowe *et al.*, Influenza vaccination responses in human systemic lupus erythematosus: impact of clinical and demographic features. *Arthritis Rheum* **63**, 2396–2406 (2011).
40. S. G. Tangye, A. Ferguson, D. T. Avery, C. S. Ma, P. D. Hodgkin, Isotype switching by human B cells is division-associated and regulated by cytokines. *J Immunol* **169**, 4298–4306 (2002).
41. S. Slight-Webb *et al.*, Autoantibody-positive healthy individuals with lower lupus risk display a unique immune endotype. *J Allergy Clin Immunol* **146**, 1419–1433 (2020).
42. J. Lubbers *et al.*, Changes in peripheral blood lymphocyte subsets during arthritis development in arthralgia patients. *Arthritis Res Ther* **18**, 205 (2016).
43. R. Z. Harms *et al.*, Abnormal T Cell Frequencies, Including Cytomegalovirus-Associated Expansions, Distinguish Seroconverted Subjects at Risk for Type 1 Diabetes. *Front Immunol* **9**, 2332 (2018).
44. N. Damond *et al.*, A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry. *Cell Metab* **29**, 755-768 e755 (2019).

45. F. Wimmers *et al.*, The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination. *Cell* **184**, 3915-3935 e3921 (2021).
46. L. Couzi *et al.*, Predominance of CD8+ T lymphocytes among periglomerular infiltrating cells and link to the prognosis of class III and class IV lupus nephritis. *Arthritis Rheum* **56**, 2362–2370 (2007).
47. A. Arazi *et al.*, The immune cell landscape in kidneys of patients with lupus nephritis. *Nat Immunol* **20**, 902–914 (2019).
48. S. Dolff *et al.*, Urinary T cells in active lupus nephritis show an effector memory phenotype. *Ann Rheum Dis* **69**, 2034–2041 (2010).
49. D. S. Singer, E. Mozes, S. Kirshner, L. D. Kohn, Role of MHC class I molecules in autoimmune disease. *Crit Rev Immunol* **17**, 463–468 (1997).
50. G. Nizzoli *et al.*, Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* **122**, 932–942 (2013).
51. N. Krishnamoorthy *et al.*, Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. *Nat Med* **14**, 565–573 (2008).
52. A. Psarras *et al.*, Functionally impaired plasmacytoid dendritic cells and non-haematopoietic sources of type I interferon characterize human autoimmunity. *Nat Commun* **11**, 6149 (2020).
53. D. H. Chae *et al.*, Telomere length predicts mortality in the Black Womens Experiences Living with Lupus (BeWELL) Study. *Journal of Immunology* **204**, (2020).
54. J. M. Pego-Reigosa *et al.*, The risk of infections in adult patients with systemic lupus erythematosus: systematic review and meta-analysis. *Rheumatology* **60**, 60–72 (2021).
55. Z. Q. Wang *et al.*, Long-Term Survival and Death Causes of Systemic Lupus Erythematosus in China A Systemic Review of Observational Studiesc. *Medicine* **94**, (2015).
56. F. Goldblatt, S. Chambers, A. Rahman, D. A. Isenberg, Serious infections in British patients with systemic lupus erythematosus: hospitalisations and mortality. *Lupus* **18**, 682–689 (2009).
57. J. Hadjadj *et al.*, Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* **369**, 718+ (2020).
58. J. R. Teijaro, Type I interferons in viral control and immune regulation. *Curr Opin Virol* **16**, 31–40 (2016).
59. A. Kusunadi *et al.*, Severely ill COVID-19 patients display impaired exhaustion features in SARS-CoV-2-reactive CD8(+) T cells. *Sci Immunol* **6**, (2021).
60. C. Maucourant *et al.*, Natural killer cell immunotypes related to COVID-19 disease severity. *Sci Immunol* **5**, (2020).
61. N. D. Beckmann *et al.*, Downregulation of exhausted cytotoxic T cells in gene expression networks of multisystem inflammatory syndrome in children. *Nat Commun* **12**, 4854 (2021).
62. R. Kurupati *et al.*, Race-related differences in antibody responses to the inactivated influenza vaccine are linked to distinct pre-vaccination gene expression profiles in blood. *Oncotarget* **7**, 62898–62911 (2016).

63. I. H. Haralambieva *et al.*, Associations between race, sex and immune response variations to rubella vaccination in two independent cohorts. *Vaccine* **32**, 1946–1953 (2014).
64. G. M. McQuillan *et al.*, Seroprevalence of measles antibody in the US population, 1999-2004. *J Infect Dis* **196**, 1459–1464 (2007).
65. C. Christy *et al.*, Effect of gender, race, and parental education on immunogenicity and reported reactogenicity of acellular and whole-cell pertussis vaccines. *Pediatrics* **96**, 584–587 (1995).
66. M. C. Hochberg, Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis and rheumatism* **40**, 1725 (1997).
67. M. Petri *et al.*, Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis and rheumatism* **64**, 2677–2686 (2012).
68. S. Slight-Webb *et al.*, Autoantibody-Positive Healthy Individuals Display Unique Immune Profiles That May Regulate Autoimmunity. *Arthritis & rheumatology*, (2016).
69. J. M. Kheir *et al.*, Unique clinical characteristics, autoantibodies and medication use in Native American patients with systemic lupus erythematosus. *Lupus science & medicine* **5**, e000247 (2018).
70. Q. Z. Li *et al.*, Risk factors for ANA positivity in healthy persons. *Arthritis research & therapy* **13**, R38 (2011).
71. A. D. Amir el *et al.*, viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat Biotechnol* **31**, 545–552 (2013).

Supplementary Materials

Materials and Methods

Fig. S1. Systems overview of overlapping samples

Fig. S2. Number of SLE-specific autoantibody specificities in EA and AA lupus disease.

Fig. S3. Whole blood tSNE plots in EA and AA patients.

Fig. S4. Altered cell frequencies by ancestry in SLE patients.

Fig. S5. Cell activation in AA and EA SLE ACT patients.

Fig. S6. AA disease activity associates with T cells versus EA with naïve B cells.

Fig. S7. EpiTOF antibody panel.

Fig. S8. Sample bioreplicates used for EpiTOF analyses.

Fig. S9. Chromatin marks significantly differentiate SLE patients from controls.

Fig. S10. Preprocessing and QC of single cell RNA-sequencing data.

Fig. S11. Harmony corrects for batch effects in scRNA-sequencing on the principal component reductions of each modality.

Fig. S12. Cell subsets identified in multimodal analysis are represented across all patients.

Fig. S13. Analysis of total single cell dataset identifies major canonical cell types.

Fig. S14. Global landscape of protein expression by CITEseq shows epitope variation by ancestry and disease status.

Fig. S15. Ingenuity Pathway Analysis of naïve B cell DEGs predicts activation of proliferation, development, and viability, and predicts inactivation of apoptosis in SLE ACT.

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Fig. S17. Ingenuity Pathway Analysis of plasmablast DEGs predicts activation of interferon-associated upstream regulators and RNA viral infection pathways in AA SLE ACT patients.

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Figures

Figure 1

Analysis of ancestry-associated differences in immune phenotypes. The workflow is broadly divided into 3 steps. First, 58 samples from EA and AA healthy controls, and SLE INACT and SLE ACT patients were matched by age, ancestry, and gender. **(A)** Whole blood was collected, left unstimulated or stimulated, and used for immunophenotyping by mass cytometry and signaling analysis by phospho-CyTOF **(B)** Plasma and supernatants collected after overnight stimulation of whole blood were collected for assessment of 39 different soluble mediators using multiplex bead-based assays and ELISAs. **(C)** PBMCs were used for droplet-based scRNA-seq and EpiTOF. For sequencing, PBMCs were washed and depleted of red blood cells and depleted of T cells using CD2 depletion to enrich for non-T cell populations. A 10X genomics platform was to examine differences in gene expression and a 51-plex CITE-seq panel was utilized to delineate specific cell lineages, activation and regulatory markers. **(D)** PBMCs from EA and AA healthy controls with no autoimmune disease manifestations were stimulated for 7 days with IFN α , TLR7/8, or TLR9 agonists, alone or in combination, to assess immune composition and antibody production by flow cytometry and ELISA, respectively.

Figure 2

Cell type composition of whole blood from EA and AA healthy controls and SLE patients with variable lupus disease activity. (A) The frequencies of major cell lineages in healthy controls, SLE INACT patients, and SLE ACT patients as determined by mass cytometry are shown. (B) tSNE marker expression plots of CD38 expression and (C) CD27 expression on T cells. The mean metal intensity (MMI) of (D) CD27 on CD8+ T cells and CD38 on (E) CD8+ T cells and (F) DN T cells were assessed from mass cytometry data

Changes in the CD8+ T cell epigenetic landscape characterize AA patients from EA and higher disease activity. PBMCs were used to assess global differences in 40 different chromatin modifications in 19 immune cell subsets. **(A)** Average marked chromatin changes for one biological replicate comparing overall changes in cell subsets of SLE patients versus controls. Heatmap depicts increased levels in red, no change in yellow, and decreased levels in blue. Examining chromatin differences by ancestry revealed marked differences in CD8+ T cells only **(B)** Scatter plot depicts the effect size comparisons of chromatin marks in CD8+ T cells between AA and EA patients. x-axis, biological replicate 1; y-axis, biological replicate 2. Each dot represents the average of a chromatin mark. Correlation coefficient and the associated p-value are shown. Normalized chromatin marker levels of EA and AA healthy controls and SLE patients for significantly altered **(C)** H3K18ac and **(D)** H3K27ac in CD8+ T cells. **(E)** ROC curves depict the segregation of CD8+ T cells from AA patients and cells from EA patients using the variance in Mark 4. Curves summarizing the results from both biological replicates are shown, with AUROC percentages from independent replicates separately listed. Classification specificity (x-axis) and sensitivity (y-axis) are shown for ROC curve. **(F)** Normalized H3K27ac levels in CD8+ T cells patients with SLE INACT and SLE ACT disease activity.

Figure 4

Alterations in the genomic landscape of SLE ACT patients reveal greater IgG frequencies in AA patients. PBMCs from 56 controls and patients with SLE were CD2 depleted and droplet-based scRNA-sequencing was done using a 10x genomics platform. **(A)** UMAP plot representing the 6 clusters of B cells. The putative identity of each cluster was determined using gene expression and protein expression from CITE-seq. **(B)** Dot plot represents expression values of selected proteins assessed by CITE-seq and a **(C)**

heatmap represents gene expression values of selected genes across each cluster used for cluster annotation. Dot size represent the percentage of cells expressing the marker of interest. Color intensity indicates the mean expression within expressing cells. Violin plots compare the proportion of each cluster across the disease groups and ancestries for **(D)** Naïve B cells, **(E)** ABCs, **(F)** Naïve IgM^{Hi} B cells, **(G)** Plasmablasts, **(H)** Transitional B cells, and **(I)** Memory B cells as defined by scRNA-seq. Ingenuity pathway analyses of differentially expressed genes identified differences in **(J)** differentiation of B cells and **(K)** transmigration genes of naïve B cells between SLE ACT disease activity patients and controls. Red indicates increased measurement of that gene expression pathway and green indicated decreased measurement. The percentage of B cells with high gene expression of class-switched IgA, IgE, IgG or IgM are shown by B cell subset in **(L)** ABCs, **(M)** GC B cells, and **(N)** Plasma cells. P values were calculated using Wilcoxon test between two disease groups. *p<0.05, and **p<0.01.

Figure 5

Monocyte ISG^{Hi} transcriptional subsets are increased in SLE ACT patients. PBMCs from 56 controls and patients with SLE were CD2 depleted and droplet-based scRNA-sequencing was done using a 10x genomics platform. **(A)** UMAP plot representing the 11 clusters of monocytes. The putative identity of each cluster was determined using gene expression and protein expression from CITE-seq. **(B)** Dot plot represents expression values of selected proteins assessed by CITE-seq and a **(C)** heatmap represents

gene expression values of selected genes across each cluster used for cluster annotation. Dot size represent the percentage of cells expressing the marker of interest. Color intensity indicates the mean expression within expressing cells. Violin plots compare the proportion of each cluster across the disease groups for (D) intermediate monocytes, (E) LYZ^{Hi}IFI6^{Hi}, (F) ISG^{Hi}, and (G) CD85^{Hi}CD94^{Hi} monocytes. (H) Gene expression for specific IFN response modules are shown using dot plot for non-classical, intermediate and classical monocytes by disease group. Violin plots compare the proportion of the three different dendritic cell populations identified by UMAP (I) Clec9a+ cDC1s (J) CD1c+ cDC2s and (K) pDCs. cDC gene expression of HLA antigen presentation components are shown via dot plot by disease group. HLA Class I markers are boxed in black and HLA Class II markers are boxed in red. P values were calculated using Wilcoxon test between two disease groups. *p<0.05, and **p<0.01.

Figure 6

TLR activation pathways are elevated with disease activity in AA patients. Differentially expressed genes between AA and EA samples identified by scRNA-seq of (A) B cells and (B) monocyte cell clusters were assessed by Ingenuity Pathway Analysis to determine differences in the activity of TLR pathways. Red

indicates increased TLR pathway activity in AA versus EA, white indicates no change, and blue indicates decreased TLR activity. **(C)** Peripheral whole blood from were stimulated for 4 or 15 minutes with IFN α , PMA and ionomycin, or TLR4, TLR7/8, or TLR9 agonists. The median 95th percentile was used to calculate the fold change of phospho-signaling and activation markers in AA versus EA controls in 8 cell populations (see legend: B cells, CD4+ T cells, CD8+ T cells, dendritic cells (DCs), plasmacytoid DCs (pDCs), natural killer (NK) cells, monocytes (mono) and granulocytes (gran)). Significant changes are noted by a blue box (decrease) or a red box (increase). $p < 0.05$

Figure 7

TLR stimulation of healthy AA and EA immune cells recapitulates ancestry-associated SLE immunophenotypes.

One million PBMCs from 10 EA and 10 AA healthy controls, devoid of any autoimmune characteristics (ANA-, RF, CCP3 antibody negative, and negative CSQ), were stimulated with R848 (TLR7/8 agonist), CpG (TLR9 agonist) or IFN α alone or in combination for 7 days. Using flow cytometry, differences in cell composition existed in cultures between EA and AA patients. **(A)** tSNE plots identified 9 different immune cell populations. **(B)** Representative tSNE plots of a healthy EA and a

healthy AA patient under the 9 different stimulation condition. CD38 expression is show with red (high expression) and blue (low expression). Cells were counted in culture following 7 day stimulation and total cell subset numbers were back-calculated using cell frequencies. The total **(C)** T cells, **(D)** NK cells, **(E)** B cells, and **(F)** myeloid cells/mL are shown for the 8 different stimulations conditions by ancestry. **(G)** B cells were further subdivided by biaxial gating on IgM and CD27 to look at 6 different B cell subsets: 1) class-switched plasmablasts, 2) IgM+ plasmablasts, 3) class-switched memory B cells, 4) IgM+ memory B cells, 5) DN B cells, and 6) naïve B cells. The frequency of **(H)** naïve B cells, **(I)** DN B cells, **(J)** class-switched memory B cells, and **(K)** class-switched plasmablasts are shown for each condition. Supernatants were collected and assessed via ELISA for **(L)** Total IgG concentrations and **(M)** IgA concentrations. *p<0.05

Figure 8

Pro-inflammatory cytokines are altered in SLE patients by ancestry and shape the TLR immune response. Pro-inflammatory soluble mediators were measured by multiplex or ELISA. Significant cytokines differences between AA (n=21) and EA (n=19) SLE patients included increased **(A)** SCF, **(B)** TNF α **(C)** BLYS, **(D)** IL-13, **(E)** IL-4, **(F)** sICAM-1 and **(G)** IL-1RA in AA patients, and increased **(H)** Eotaxin in EA patients. Linear regression analyses show **(I)** SCF and **(J)** MCP-1 to increase with lupus disease

activity (n=40). **(K)** A heatmap summary of the supernatant levels following 24 hour whole blood stimulation with TLR4/7/8/9 agonists for each disease group are shown. Soluble mediator levels are displayed on a color scale ranging from blue (protein levels below the mean) through white (protein levels equal to the mean) to red (protein levels greater than the mean) using a column z-score. The most significant fold change differences over unstimulated culture samples were in the IFN pathways including **(L)** IFN γ , **(M)** IL-18, and **(N)** IFN α . TLR-stimulated culture supernatant levels of IFN α negatively associated with ISG gene expression modules in **(O)** classical and **(P)** non-classical monocytes by linear regression analysis. *p<0.05, **p<0.01. Kruskal-Wallis test with two-tailed Mann-Whitney for multiple comparisons. Mean $\pm$ SD shown.

Supplementary Files

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