Published in final edited form as:

J Autoimmun. 2015 June ; 60: 51–58. doi:10.1016/j.jaut.2015.04.002.

Widely Divergent Transcriptional Patterns Between SLE Patients of Different Ancestral Backgrounds in Sorted Immune Cell Populations

Shruti Sharma^{#1}, Zhongbo Jin^{#2}, Elizabeth Rosenzweig¹, Swapna Rao¹, Kichul Ko¹, and Timothy B. Niewold²

¹University of Chicago, Gwen Knapp Center for Lupus Research

²Mayo Clinic, Division of Rheumatology and Department of Immunology

[#] These authors contributed equally to this work.

Abstract

Systemic lupus erythematosus (SLE) is a complex autoimmune disease of uncertain etiology. Patients from different ancestral backgrounds demonstrate differences in clinical manifestations and autoantibody profiles. We examined genome-wide transcriptional patterns in major immune cell subsets across different ancestral backgrounds. Peripheral blood was collected from African-American (AA) and European-American (EA) SLE patients and controls. CD4 T-cells, CD8 T-cells, monocytes, and B cells were purified by flow sorting, and each cell subset from each subject was run on a genome-wide expression array. Cases were compared to controls of the same ancestral background. The overlap in differentially expressed gene (DEG) lists between different cell types from the same ancestral background was modest (<10%), and only 5-8% overlap in DEG lists was observed when comparing the same cell type between different ancestral backgrounds. IFN-stimulated gene (ISG) expression was not up-regulated synchronously in all cell types from a given patient, for example a given subject could have high ISG expression in T and B cells, but not in monocytes. AA subjects demonstrated more concordance in ISG expression between cell types from the same individual, and AA patients demonstrated significant down-regulation of metabolic gene expression which was not observed in EA patients. ISG expression was significantly decreased in B cells in patients taking immunosuppressants, while ISGs in other cell types did not differ with medication use. In conclusion, gene expression was strikingly different between immune cell subsets and between ancestral backgrounds in SLE patients. These findings emphasize the critical importance of studying multiple ancestral backgrounds and multiple cell types in gene expression studies. Ancestral backgrounds which are not studied will not benefit from personalized medicine strategies in SLE.

© 2015 Published by Elsevier Ltd.

Correspondence: Timothy B. Niewold, MD, Mayo Clinic, Division of Rheumatology and Department of Immunology, 200 1st St. SW, Guggenheim Bld. 3-42, Rochester, MN, 55905. Phone: (507) 284 8450. Fax: (507) 284 0564. niewold.timothy@mayo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Financial Disclosures and Conflict of Interest: The authors report no financial conflict of interest.

Keywords

Systemic Lupus Erythematosus; Interferon; Ancestral Background; Gene Expression

1. Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by multi-system organ involvement, autoantibody production, and tissue injury. The etiology involves a combination of multiple genetic and environmental factors whose amalgamation breaches the threshold of immune tolerance. Clinically, SLE patients from different ancestral backgrounds manifest differently. For example, Amerindian genetic ancestry is associated with earlier onset of SLE and an increased risk of developing renal involvement as compared to European-American ancestry [1]. Distinct patterns of genetic association with SLE exist between world populations, with some genetic factors being associated in one background but not the other [2-6]. These data support the idea that biological differences between populations underlie the well-described differences in clinical manifestations.

Type I IFN is a primary pathogenic factor in human SLE [7-10]. IFN- α serum levels are elevated in SLE patients and correlate with disease activity [11, 12]. Serum IFN- α levels are higher in African-American SLE patients than in European-American SLE patients on average [13]. Mirroring the serum data, expression of IFN- α -stimulated genes (ISGs) are up-regulated in SLE patient peripheral blood mononuclear cells (PBMCs) as compared with healthy individuals [14-16]. This increased ISG expression also correlates with greater disease activity and particular autoantibody profiles and disease phenotypes in SLE patients [12]. Moreover, this up-regulation of ISG expression may be useful in distinguishing between different autoimmune diseases and other febrile conditions [17]. Thus, transcriptional programs in circulating blood cells from SLE patients and other conditions can provide an important window into disease pathogenesis.

The over-representation of type I IFN-induced transcripts in peripheral blood cells from patients with SLE and other autoimmune conditions has been documented and replicated in numerous previous studies [18, 19]. These studies were carried out in either whole blood [20, 21], or peripheral blood mononuclear cells (PBMCs) [14, 15, 22]. However, both whole blood and PBMC are mixture of different cell subsets which are present in different proportions in different people. Because each of the contributing cell types expresses a somewhat unique gene expression signature relating to its function, the relative proportions of cell types present in a given sample affects the gene expression profile [23]. Therefore, when differences are detected between people, it is possible that either a particular transcript was over- or under-expressed uniformly in a majority of the cell types, or that the transcript was expressed at the same level in a particular cell subset which was more numerous in one person as compared to the other. These differences are not easy to resolve, as it is likely that both of these examples are occurring at the same time to differing degrees with different transcripts. Investigating gene expression profiles in homogeneous cell populations might avoid this confounding and may exhibit stronger power to depict disease pathogenesis. To

date, some studies have examined a single immune cell population in autoimmune disease [24-26], and two studies have used multiple homogeneously isolated immune cells from SLE patients and healthy controls [27, 28]. These studies have begun to address this complexity, and in the current study we extend these comparisons to major PBMC subsets in a large number of SLE cases (3 times larger than previous studies [27, 28]), allowing for comparison of subjects from two major ancestral backgrounds, African-American (AA) and European-American (EA).

2. Methods

2.1 Subjects and samples

Blood samples from SLE patients and controls from both AA and EA ancestry were obtained from the University of Chicago Medical Center (UCMC). Only female subjects were included in this study. Twenty one patients and five controls were included from each ancestral background (Table 1). All the cases fulfilled the American College of Rheumatology criteria for the diagnosis of SLE [29, 30]. The controls were screened by the authors (TBN) for the absence of autoimmune diseases. All the subjects provided informed consent, and the study was approved by the institutional review board. SLEDAI scores were assessed at the time of blood draw for both the AA and EA patients.

2.2 Isolation of immune cell types, RNA preparation, and microarray experiment

PBMCs were isolated from peripheral blood samples by using a Ficoll-Paque™ gradient (Amersham). PBMCs were sorted by flow cytometry using the following staining antibodies: CD4-FITC, CD8- PE, CD14-PerCP and CD20-APC to isolate purified populations of CD4+ T-cells, CD8+ T-cells, monocytes and B cells. From each of the purified cell populations, RNA was isolated from each of the purified cell populations, and analyzed on an Agilent Bioanalyser to calculate RNA Integrity Number (RIN) scores. RNA amplification was performed on all samples using TargetAmp™ 2-Round Biotin-aRNA Amplification Kit 3.0 from Epicentre. To minimize technical variation throughout sample handling and processing, we processed different cell subsets from the two ancestries in the same batches to reduce the potential contribution of batch effect to the cross-ancestral background comparisons. The cRNA was prepared in large batches (48 samples) including all cell types and both ancestral backgrounds, and all the cell subsets from the same patient were processed in the same batch to enable between cell-type comparisons. The arrays were run in the Microarray Core Facility at the University of Chicago. The Labeled cRNA was hybridized overnight to HumanHT-12 V4 expression BeadChip arrays (Illumina, San Diego, CA), washed, blocked, stained and scanned on an Illumina BeadStation (HiScan). The background measured on the arrays and detection of internal hybridization controls were used as standard quality control metrics. The dataset described in this manuscript has been deposited in the GEO database.

2.3 Microarray data extraction and normalization

Illumina's Genome Studio (Gene expression module 1.9.0) software was used to generate signal intensity values and detection P values from the scans. Only the probes with average signal intensity value greater than 0.1 and a detection p value less than 0.05 were considered

present in this study. The raw data was then filtered by creating a PALO (present in at least one sample) list for the entire study (208 arrays). Of the 44,589 probes on the bead-arrays, 38,932 probes were on the PALO list. Quantile normalization followed by $\log_2$ transformation was applied to the PALO list for the entire study using Partek Genomics Suite 6.6.

2.4 Analysis of differentially expressed genes (DEGs)

Samples were grouped by cell type, ancestry, and case vs. control status. The groups were labeled as CD4 AA, CD4 EA, etc. There were 8 study groups in total; each of which contained samples from 21 samples and 5 controls. After normalization, a PALO list was created for each study group, and a two tailed unequal variance t-test was used to identify DEGs with a threshold p-value of <0.05 . To study the similarities of DEGs from each study group, we calculated the percentage of probes which were common between different DEGs lists from different groups. Additionally, we used Ingenuity Pathway Analysis (IPA) software to detect the top dysregulated canonical pathways (Ingenuity Systems, www.ingenuity.com). Venn diagrams and hierarchical clustering plots were generated using Partek genomics suite 6.6.

2.5 Method for Quantitative Assessment of ISG Expression

To study ISG expression, we generated a comprehensive list from the published literature which included 483 ISGs [14, 31-37]. Our ISG list corresponded to 737 probes on the Illumina arrays. From them, 606 probes were detected in at least one of 208 samples (Supplemental table 1). For each ISG, we calculated the difference in quantitative expression between each subject and the average of matched controls. Then, the sum of the differences of all ISGs was calculated for each cell subset in each patient. If the sum was greater than zero, then ISG expression was considered up-regulated. Subjects were also assigned a rank from 1 to 21 from highest to lowest ISG expression for each cell subset. Previous studies show that IFN signatures in PBMCs from SLE patients were significantly down-regulated after treatment with high dose intravenous methylprednisolone [14]. To analyze the impact of medication use, we separated SLE patients into two treatment categories: patients who took Plaquenil +/- low dose oral prednisone or NSAIDs, or patients who took immunosuppressant such like AZA, MMF, etc. with or without plaquenil. Only one patient (AA subject) was not taking any medications. ISG ranks were compared between treatment groups in each cell type and in each ancestral background.

2.6 PCR Validation of microarray findings

We validated 46 genes in the monocyte cell type from the African-American cases. First, cDNAs were synthesized from original RNA samples, then target gene pre-amplification was carried out before an rt-PCR reaction on a 48x48 microfluidic PCR array using the Fluidigm Biomark HD PCR system. We first evaluated the correlation of microarray data and rt-PCR data per subjects. The overall expression level at these 46 genes measured as the sum of the delta-CT values was significantly correlated with the sum of the normalized microarray intensity values for these same 46 genes (Supplemental Figure 1A), with a p-value of 0.038. When individual genes were examined, delta-CT values were also

significantly correlated with microarray intensity values (two examples are shown in Supplemental Figure 1B and 1C), for example, IFI27 correlation p-value of 0.0010).

2.7 Control for genetic ancestry and admixture

To control for genetic ancestry and admixture, we genotyped 332 ancestry-informative markers (AIMs) designating continental ancestry in all of our cases and controls, as described in [38]. The AIMs data were analyzed using principal component analysis, and the first principal component designated African vs. non-African ancestry, as would be expected. Comparing the principal component values generated from the AIMs, we did not appreciate any population outliers, and there were no significant differences between the cases and controls in either ancestral background (Supplemental Figure 2). These results demonstrate that the admixture proportions were similar in our African-American cases and controls, and thus differences in admixture or unappreciated discordance between self-reported and genetic ancestry should not be confounding the results.

3. Results

3.1 Modest overlap among DEG lists of different cell types and different ancestral backgrounds

The total number of DEGs was higher in AA SLE patients than in EA SLE patients, and this difference was observed across all cell populations ($p=2.2 \times 10^{-53}$ by Chi-square distribution comparing proportions of AA DEGs vs. EA DEGs, Figure 1A). Surprisingly, the overlap in DEG lists between different cell types within the same ancestral background was modest (<10%, Figure 1B), suggesting large differences in the transcriptional profile of major immune cell lineages. For example, there were 200 DEGs in common between CD4 AA and CD8 AA, with an overlap rate of 7.91%. In support of this finding, principal component analysis (PCA) of the gene expression data set supports clustering of the major cell types as the most important component of variance (Figure 1C). The two T cell subsets showed more overlap than other cell types, and these two cell types clustered more closely in the PCA analysis. We also conducted comparisons across ancestral backgrounds, and a similarly low rate of overlap was observed between ancestral backgrounds when comparing cells of the same type (7.99% to 4.87%, Figure 1D).

3.2 Cell-type specific gene expression profiles of different subjects exhibited individualized patterns on unsupervised hierarchical clustering

To reveal the relationships between gene expression patterns from individual controls and patients, we performed unsupervised hierarchical clustering of each cell type (Figure 2). The majority of the control samples were clustered into two branches, which belonged to AA and EA ancestry respectively. These two branches within the controls have a short distance on the clade diagrams, suggesting that differences between control groups, even across ancestral backgrounds were less than that observed between the controls and the cases. The patients' profiles showed cell-type independent inter-individual variations, and no obvious separation was visualized between the two ancestral backgrounds (Supplemental table 2). Similarly, we did not find any groups within the various cell types which are defined by treatment. For example, subjects treated with hydroxychloroquine alone were not clustered

separately from those subjects taking immunosuppressive agents. The strikingly different and individual patterns demonstrated in the SLE patient profiles suggest a wide diversity of immune cell function within different SLE patients.

3.3 Energy metabolism pathways were down-regulated in all cell types from AA ancestry

We used pathway analysis to determine which pathways were over-represented in our DEG lists from each study group. Based on biological function, these pathways can be assigned into 3 categories, energy metabolism, cell death/survival/cell cycle, and immune related pathways (Supplemental Table 3). In particular, the glycolysis and gluconeogenesis pathways were in the top list from all AA patient cell types except that gluconeogenesis was not in the top list of CD8 AA. Both of these metabolic pathways were significantly down regulated. Interestingly, significant dysregulation of these two pathways in EA patients was not observed. Pathways related to cell cycle/cell death were common in multiple cell type lists from EA groups. For example, “Role of CHK Proteins in Cell Cycle Checkpoint Control” was a common over-represented pathway across three EA ancestry cell subsets (CD4, CD8, and CD20).

3.4 Diversity in ISG up-regulation between ancestral backgrounds and cell types

In order to analyze ISG expression comprehensively, we created quantitative metric to assess global ISG expression (See Methods section). First we evaluated the expression level of these ISGs in control groups. There was no significant difference in the number of expressed ISGs in AA controls compared with EA controls ($p>0.05$). For each cell type, we summed up all expressed ISG signal values; this represented the magnitude of ISG expression in each subject. There was no significant difference between AA controls and EA controls comparing ISG expression ($p>0.05$). We then analyzed ISG expression in different cell types in AA and EA study groups. The number of differentially expressed ISGs was significantly greater in AA subjects than in EA subjects, similar to previous studies [13, 39] (36 to 74 out of 606 in AA groups, and 28 to 49 out of 606 in EA groups). Overlap in ISGs between cell types was modest, similar to the overlap rate in overall DEGs (Tables 2 and 3). Only two genes, IFI27 and ALDOC, were in common in all cell types in AA patients. Similarly, ISG overlap between ancestral backgrounds in the same cell type was also modest (Table 4). These data support striking diversity in the IFN response in different cell types and different ancestral backgrounds.

3.5 ISG expression was more concordant across cell types in AA patients than EA patients

Next, we evaluated ISG expression in cell types from individual SLE patients, ranked by strength of ISG over-expression (from 1-21). The ranking results are summarized in Supplemental Table 4, and rank-order concordance among cell types is summarized in Supplemental Table 5. Somewhat surprisingly, the concordance of ISG expression in different immune cell types differed widely between patients, and many patients demonstrated up-regulation of ISGs in only one or two cell populations (Figure 3). Based on the patterns of ISG concordance in different immune cells in the same person, we were able to identify subgroups of patients. One subgroup had up-regulated ISG expression in all four immune cell types, and included 6 out of 21 AA subjects and 2 out of 21 EA subjects. Another

subgroup had increased IFN-induced gene expression in both T and B cells but not monocytes, and included 10 AA subjects and 11 EA subjects.

3.6 Correlation between medication use and ISG expression

We summed the ISG ranks from all four cell types in each subject to represent the relative strength of overall ISG response in PBMCs as compared to the other patients in the study. When we compared these overall PBMC ISG ranks between patients not taking immunosuppressants to those taking immunosuppressants, there were no significant differences between these two categories of medication use (Figure 4 A & B, see Methods for definition of medication categories). When we studied each immune cell type separately, we found that ISG expression was significantly reduced in B cells from AA patients taking immunosuppressants as compared to B cells of AA patients not taking immunosuppressants (p value 0.0081, Figure 4C). A similar non-significant trend was observed in B cells from EA subjects (Figure 4D). The analysis was significant when subjects of both ancestral backgrounds were combined (p value 0.010, Figure 4E). Interestingly, there were no significant changes in other cell types in relation to the medication data.

3.7 Comparison of Genetic Ancestry, SLEDAI score, and ISG expression

To rule out the contribution of admixture differences in AA population, we genotyped ancestry informative markers in the cases and controls, as described in the Methods section. There were no differences in African admixture observed between AA cases and controls (Supplemental Figure 2). We also compared the quantitative values expressing the degree of African vs. European ancestry (principal component 1 values as shown in Supplemental Figure 2) with expression levels of DEGs in all 4 cell subsets, and we did not find any significant correlations between DEG expression level and the degree of West African ancestry ($p > 0.05$ for all correlations).

Regarding SLE disease activity, we did not find significant differences between the SLEDAI scores of the African-American and European-American SLE patients included in the study (Mann-Whitney U-test $p = 0.58$). We compared ISG expression in high vs. low SLEDAI SLE patients, to detect differences in cell-type specific IFN scores based upon SLEDAI score. We divided the cases from each ancestral background into two groups, based on a cutoff SLEDAI score of 6 or greater. Within each cell type group, we compared IFN scores between patients with active disease and the patients in remission. We did not find any significant differences of IFN scores between these two SLEDAI score categories ($p > 0.05$ for all cell types, both ancestral backgrounds). Further supporting this idea, we looked for correlation between SLEDAI and IFN score in each ancestral background, and did not observe any correlation in any cell type ($p > 0.05$ for all).

4. Discussion

Gene expression profiling of peripheral blood cells has been a useful tool in studying human SLE, but study of whole blood or mixed PBMC populations can be confounded by variation in relative proportions of the cell populations between individuals. In this study, we found unique patterns of gene expression in CD4 T cells, CD8 T cells, B cells and monocytes in

our SLE patients, with little overlap of DEG lists between the different cell populations. This finding is consistent with a previous smaller study where distinct patterns were observed in SLE peripheral blood B cells, CD4 T cells, and myeloid cells[20]. In this previous study, only 4.4% of transcripts were shared across all the three cell subsets and 10% of transcripts were shared between B and T cells. This study did not assess CD8 T cells or purified monocytes, and also did not provide ancestral background information for their subjects. Our PCA results also reinforced the relevance of studying these subsets separately. The two T cell subsets were clustered together more closely than the other subsets in both AA and EA cases in PCA plots, reflecting the greater similarity in lineage in the two T cell populations.

The top canonical pathways from the DEG list in each study group were interesting. Two metabolic pathways, glycolysis and gluconeogenesis, were down regulated specifically in AA cases. In vitro studies have demonstrated that SLE T-cells are less efficient at ATP generation [40]. Additionally, metabolic profiling studies in SLE patient blood samples have demonstrated a large decrease in many different energy generating pathways, including glycolysis [41]. The majority of the SLE patients included in this metabolomics study were of AA and Hispanic-American ancestry. In another study, lower mRNA expression of the genes encoding glycolytic enzymes has been shown in the leukocytes of Asian SLE patients as compared to controls [42]. It is currently unclear why this reduction in metabolic pathway activation in SLE blood is observed, but it has been independently shown in a number of studies, including the present one. Our study would suggest that there may be ancestral differences in this decreased metabolic pathway activation in SLE patients, as it was not shared by EA patients, and other studies supporting this phenomenon have largely been in non-European ancestry SLE populations.

AA ancestral background has been associated with increased incidence and severity of disease, and increased levels of autoantibodies and type I IFN [1][13, 39, 43]. In the present study, we saw a greater number of DEGs in AA patients as compared to EA patients in each cell type. We did not find differences in ISG expression based upon SLEDAI score, but it is possible that the increased number of DEGs observed in AA cases may relate to increased disease severity in this ancestral background. The overlap of these DEGs between AA and EA subjects was modest, even when comparing the same cell type between ancestral backgrounds. When examining IFN pathway genes represented in our study, we also found differences in ISG expression between the two ancestral backgrounds, supporting diversity in IFN pathway activation between different ancestral backgrounds which has been suggested by our previous studies [13, 39, 43]. We found that different ISGs were expressed in different cell types, supporting the idea that each cell type contributes a particular panel of genes to the previously reported overall PBMC or whole blood IFN signature. Presumably this diversity in ISG expression reflects some of the different cellular mechanisms and functions of type I IFN in the various peripheral blood cells. Strikingly, we found that within a given individual, particular cell subsets had elevated ISG expression while other cell subsets from the same blood sample did not. The patterns of cell types with high ISG expression also differed by ancestry, with greater concordance of ISG expression between cell subsets observed in AA as compared to EA subjects. It seems that this distinction will have biological relevance in the immune system – which cells are being acted upon by type I

IFN in this particular patient? It is possible that grouping SLE patients by these patterns of ISG expression within cell types could be useful in predicting clinical outcomes or responses to therapy. It is possible that up-regulation of ISGs in monocytes vs. lymphocytes results in different clinical features of disease, or that anti-IFN therapy might behave differently depending upon which cell subsets are more highly activated by IFN. These findings underscore the importance of studying multiple ancestral backgrounds in human SLE.

We observed an impact of immunosuppressive therapy on ISG expression in peripheral blood, but this was only present in B cells, and the signal was lost when all cell types were averaged together. It is hard to speculate regarding the molecular mechanism by which ISG expression would be suppressed in B cells specifically, but this finding reinforces the need to study immune cell subsets individually, as combined data may obscure findings from specific cell populations. Future in vitro experiments could be considered to test whether this is a direct or secondary effect, etc. And ideally a prospective study comparing B cell ISG expression before and after different immunosuppressive therapies would be desired. There are some limitations to the present study. We did not have access to data regarding some aspects of disease, such as duration of disease. This factor may or may not correlate with some of the findings we describe in the present study. We were able to examine disease activity in the patients, and the differences observed in ISG expression were not correlated with disease activity as measured by the SLEDAI score. It would also be interesting to study the association of genetic variations with gene expression level of SLE associated genes. This could improve our understanding of the impact of SLE-associated genetic variations in human SLE pathogenesis.

5. Conclusions

In conclusion, gene expression was strikingly different between immune cell subsets within the same ancestral background, and in the same immune cell type when compared between ancestral backgrounds in SLE patients. This suggests that diverse biological mechanisms contribute to disease in different ancestral backgrounds (for example, reduced metabolic transcriptional activity in AA but not EA patients). ISG expression was diverse between cell types in the same person, and treatment with the immunosuppressive agents correlated with the specific down-regulation ISGs in B cells. These findings emphasize the critical importance of studying multiple ancestral backgrounds and multiple cell types in gene expression studies. Ancestral backgrounds which are not studied will not benefit from personalized medicine strategies in SLE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: K Ko – Arthritis Foundation Clinical to Research Transition Award; TB Niewold – Research grants from the NIH (AR060861, AR057781, AR065964, AI071651), Lupus Foundation of Minnesota, American College of Rheumatology Research Foundation, and the Mayo Clinic Foundation.

References

1. Sanchez E, Rasmussen A, Riba L, Acevedo-Vasquez E, Kelly JA, Langefeld CD, et al. Impact of genetic ancestry and sociodemographic status on the clinical expression of systemic lupus erythematosus in American Indian-European populations. *Arthritis Rheum.* 2012; 64:3687–94. [PubMed: 22886787]
2. Ramos PS, Oates JC, Kamen DL, Williams AH, Gaffney PM, Kelly JA, et al. Variable association of reactive intermediate genes with systemic lupus erythematosus in populations with different African ancestry. *J Rheumatol.* 2013; 40:842–9. [PubMed: 23637325]
3. Sanchez E, Comeau ME, Freedman BI, Kelly JA, Kaufman KM, Langefeld CD, et al. Identification of novel genetic susceptibility loci in African American lupus patients in a candidate gene association study. *Arthritis and rheumatism.* 2011; 63:3493–501. [PubMed: 21792837]
4. Pothlichet J, Niewold TB, Vitour D, Solhonne B, Crow MK, Si-Tahar M. A loss-of-function variant of the antiviral molecule MAVS is associated with a subset of systemic lupus patients. *EMBO Mol Med.* 2011; 3:142–52. [PubMed: 21268286]
5. Lodolce JP, Kolodziej LE, Rhee L, Kariuki SN, Franek BS, McGreal NM, et al. African-derived genetic polymorphisms in TNFAIP3 mediate risk for autoimmunity. *J Immunol.* 2010; 184:7001–9. [PubMed: 20483768]
6. Ghodke-Puranik Y, Niewold TB. Genetics of the type I interferon pathway in systemic lupus erythematosus. *Int J Clin Rheumatol.* 2013;8.
7. Niewold TB. Interferon alpha as a primary pathogenic factor in human lupus. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research.* 2011; 31:887–92.
8. Shrivastav M, Niewold TB. Nucleic Acid Sensors and Type I Interferon Production in Systemic Lupus Erythematosus. *Frontiers in immunology.* 2013; 4:319. [PubMed: 24109483]
9. Niewold TB, Hua J, Lehman TJ, Harley JB, Crow MK. High serum IFN-alpha activity is a heritable risk factor for systemic lupus erythematosus. *Genes Immun.* 2007; 8:492–502. [PubMed: 17581626]
10. Kariuki SN, Franek BS, Kumar AA, Arrington J, Mikolaitis RA, Utset TO, et al. Trait-stratified genome-wide association study identifies novel and diverse genetic associations with serologic and cytokine phenotypes in systemic lupus erythematosus. *Arthritis Res Ther.* 2010; 12:R151. [PubMed: 20659327]
11. Kim T, Kanayama Y, Negoro N, Okamura M, Takeda T, Inoue T. Serum levels of interferons in patients with systemic lupus erythematosus. *Clin Exp Immunol.* 1987; 70:562–9. [PubMed: 2449306]
12. Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. *Arthritis Rheum.* 2005; 52:1491–503. [PubMed: 15880830]
13. Weckerle CE, Franek BS, Kelly JA, Kumabe M, Mikolaitis RA, Green SL, et al. Network analysis of associations between serum interferon-alpha activity, autoantibodies, and clinical features in systemic lupus erythematosus. *Arthritis Rheum.* 2011; 63:1044–53. [PubMed: 21162028]
14. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med.* 2003; 197:711–23. [PubMed: 12642603]
15. Kirou KA, Lee C, George S, Louca K, Papagiannis IG, Peterson MG, et al. Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus. *Arthritis Rheum.* 2004; 50:3958–67. [PubMed: 15593221]
16. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A.* 2003; 100:2610–5. [PubMed: 12604793]
17. Pascual V, Allantaz F, Patel P, Palucka AK, Chaussabel D, Banchereau J. How the study of children with rheumatic diseases identified interferon-alpha and interleukin-1 as novel therapeutic targets. *Immunol Rev.* 2008; 223:39–59. [PubMed: 18613829]
18. Mandel M, Achiron A. Gene expression studies in systemic lupus erythematosus. *Lupus.* 2006; 15:451–6. [PubMed: 16898181]

19. Ronnblom L, Eloranta ML. The interferon signature in autoimmune diseases. *Curr Opin Rheumatol.* 2013; 25:248–53. [PubMed: 23249830]
20. Higgs BW, Zhu W, Richman L, Fiorentino DF, Greenberg SA, et al. Identification of activated cytokine pathways in the blood of systemic lupus erythematosus, myositis, rheumatoid arthritis, and scleroderma patients. *Int J Rheum Dis.* 2012; 15:25–35. [PubMed: 22324944]
21. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature.* 2010; 466:973–7. [PubMed: 20725040]
22. Kawasaki M, Fujishiro M, Yamaguchi A, Nozawa K, Kaneko H, Takasaki Y, et al. Fluctuations in the gene expression of peripheral blood mononuclear cells between the active and inactive phases of systemic lupus erythematosus. *Clin Exp Rheumatol.* 2010; 28:311–7. [PubMed: 20460036]
23. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, et al. Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci U S A.* 2003; 100:1896–901. [PubMed: 12578971]
24. McKinney EF, Lyons PA, Carr EJ, Hollis JL, Jayne DR, Willcocks LC, et al. A CD8+ T cell transcription signature predicts prognosis in autoimmune disease. *Nat Med.* 2010; 16:586–91. 1p following 91. [PubMed: 20400961]
25. Garaud JC, Schickel JN, Blaison G, Knapp AM, Dembele D, Ruer-Laventie J, et al. B cell signature during inactive systemic lupus is heterogeneous: toward a biological dissection of lupus. *PLoS One.* 2011; 6:e23900. [PubMed: 21886837]
26. Kawasaki M, Fujishiro M, Yamaguchi A, Nozawa K, Kaneko H, Takasaki Y, et al. Possible role of the JAK/STAT pathways in the regulation of T cell-interferon related genes in systemic lupus erythematosus. *Lupus.* 2011; 20:1231–9. [PubMed: 21980035]
27. Becker AM, Dao KH, Han BK, Kornu R, Lakhanpal S, Mobley AB, et al. SLE Peripheral Blood B Cell, T Cell and Myeloid Cell Transcriptomes Display Unique Profiles and Each Subset Contributes to the Interferon Signature. *PLoS One.* 2013; 8:e67003. [PubMed: 23826184]
28. Lyons PA, McKinney EF, Rayner TF, Hatton A, Woffendin HB, Koukoulaki M, et al. Novel expression signatures identified by transcriptional analysis of separated leucocyte subsets in systemic lupus erythematosus and vasculitis. *Ann Rheum Dis.* 2010; 69:1208–13. [PubMed: 19815495]
29. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997; 40:1725. [PubMed: 9324032]
30. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1982; 25:1271–7. [PubMed: 7138600]
31. Santer DM, Wiedeman AE, Teal TH, Ghosh P, Elkon KB. Plasmacytoid dendritic cells and C1q differentially regulate inflammatory gene induction by lupus immune complexes. *J Immunol.* 2012; 188:902–15. [PubMed: 22147767]
32. van Baarsen LG, Wijbrandts CA, Rustenburg F, Cantaert T, van der Pouw Kraan TC, Baeten DL, et al. Regulation of IFN response gene activity during infliximab treatment in rheumatoid arthritis is associated with clinical response to treatment. *Arthritis Res Ther.* 2010; 12:R11. [PubMed: 20096109]
33. Guiducci C, Gong M, Xu Z, Gill M, Chaussabel D, Meeker T, et al. TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus. *Nature.* 2010; 465:937–41. [PubMed: 20559388]
34. Zimmerer JM, Lesinski GB, Ruppert AS, Radmacher MD, Noble C, Kendra K, et al. Gene expression profiling reveals similarities between the in vitro and in vivo responses of immune effector cells to IFN- α . *Clin Cancer Res.* 2008; 14:5900–6. [PubMed: 18794103]
35. Pos Z, Selleri S, Spivey TL, Wang JK, Liu H, Worschech A, et al. Genomic scale analysis of racial impact on response to IFN- α . *Proc Natl Acad Sci U S A.* 2010; 107:803–8. [PubMed: 20080756]

36. Hervás-Stubbs S, Perez-Gracia JL, Rouzaut A, Sanmamed MF, Le Bon A, Melero I. Direct effects of type I interferons on cells of the immune system. *Clin Cancer Res*. 2011; 17:2619–27. [PubMed: 21372217]
37. Yao Y, Richman L, Higgs BW, Morehouse CA, de los Reyes M, Brohawn P, et al. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. *Arthritis Rheum*. 2009; 60:1785–96. [PubMed: 19479852]
38. Kariuki SN, Ghodke-Puranik Y, Dorschner JM, Chrabot BS, Kelly JA, Tsao BP, et al. Genetic analysis of the pathogenic molecular sub-phenotype interferon-alpha identifies multiple novel loci involved in systemic lupus erythematosus. *Genes and immunity*. 2015; 16:15–23. [PubMed: 25338677]
39. Ko K, Koldobskaya Y, Rosenzweig E, Niewold TB. Activation of the Interferon Pathway is Dependent Upon Autoantibodies in African-American SLE Patients, but Not in European-American SLE Patients. *Frontiers in immunology*. 2013; 4:309. [PubMed: 24101921]
40. Fernandez D, Perl A. Metabolic control of T cell activation and death in SLE. *Autoimmun Rev*. 2009; 8:184–9. [PubMed: 18722557]
41. Wu T, Xie C, Han J, Ye Y, Weiel J, Li Q, et al. Metabolic disturbances associated with systemic lupus erythematosus. *PLoS One*. 2012; 7:e37210. [PubMed: 22723834]
42. Lee HT, Lin CS, Lee CS, Tsai CY, Wei YH. Increased 8-hydroxy-2'-deoxyguanosine in plasma and decreased mRNA expression of human 8-oxoguanine DNA glycosylase 1, antioxidant enzymes, mitochondrial biogenesis-related proteins and glycolytic enzymes in leukocytes in patients with systemic lupus erythematosus. *Clin Exp Immunol*. 2013
43. Ko K, Franek BS, Marion M, Kaufman KM, Langefeld CD, Harley JB, et al. Genetic ancestry, serum interferon-alpha activity, and autoantibodies in systemic lupus erythematosus. *J Rheumatol*. 2012; 39:1238–40. [PubMed: 22505704]

Highlights

Gene expression differed between ancestral backgrounds and immune cell subsets

African-American patients demonstrated down-regulation of metabolic transcripts

Interferon signature was diverse between cell types in the same patient

Interferon-induced genes were reduced in B cells of patients on immunosuppressants

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Analysis of DEGs in different cell subsets with AA or EA ancestral background. A. shows the number of DEGs in each study group, with up-regulated DEGs indicated in red and down-regulated DEGs indicated in blue. B. PCA plot of gene expression data color-coded by cell type, red CD4 T cells; blue CD8 T cells, green CD14 monocytes, dark purple CD20 B cells; C. Venn diagram of the DEGs for each cell subset with percentage overlap indicated for each ancestral background. D. shows overlap between EA and AA ancestral backgrounds in each cell type, green circles represents AA DEGs and the pink circles represent EA DEGs.

Figure 2.

Unsupervised hierarchical clustering of DEG lists of each cell subsets. The subjects are arranged vertically, and each cell type is represented in a different diagram. Within each cell-type diagram, column 1 indicates ancestral background: blue = EA patients, red = AA patients, light pink = AA controls, light green = EA controls. In the second column a different color is used to represent each subject. Heatmap was generated by using Partek genomics suite 6.6.

Figure 3.

IFN signatures among different immune cell types in both ancestral backgrounds. Relative strength of ISG expression by cell type is shown in a multi-layer donut graph. The concentric circles each represent a different cell type, from inner to outer rings: CD4, CD8, CD20 and CD14. Each subject is represented as a wedge from inside to outside. Increased red shading indicates increased ISG expression, and green indicates no increased ISG expression.

Figure 4.

Correlation between medication use and IFN-induced gene expression. A. shows a comparison of overall IFN rank from PBMC in EA SLE patients, stratified by treatment, and B. shows the same analysis in AA SLE patients. C. shows a comparison of ISG rank scores in each cell type separately, stratified by treatment group in AA SLE patients, D. shows the same analysis in EA SLE patients, and E. shows the same analysis in both ancestral backgrounds combined. Plaq = patients who were taking Plaquenil +/- low dose oral prednisone or NSAIDs, Imm = patients who were taking immunosuppressants.

Subjects

Table 1

	SLE cases	Mean Age in years (SD)	Median SLEDAI	Interquartile range SLEDAI	% on oral Immuno-suppressants	% on hydroxy-chloroquine +/- low dose steroid
African-American(AA)	21	43.0 (13.4)	4	0 to 8	42.8	47.6*
European-American(EA)	21	43.1 (12.5)	4	2 to 6	61.9	38.1

There was no difference in age amongst the groups.

All the cases fulfilled the ACR criteria for the diagnosis of SLE.

* Data regarding medication use was not available for one AA SLE patient, so total adds to less than 100%

Table 2
Degree of overlap in DE-ISG between different cell subsets in AA ancestry

# of DE-ISG in common / overlap rate			
AA	CD8	CD14	CD20
CD4	5 / 6.58%	8 / 7.27%	7 / 6.73%
CD8		10 / 8.77%	11 / 10.19%
CD14			10 / 7.04%

Table 3
Degree of overlap in DE-ISG between different cell subsets in EA ancestry

# of DE-ISG in common / overlap rate			
	CD8	CD14	CD20
CD4	8 / 12.5%	3 / 3.53%	3 / 4.62%
CD8		1 / 1.30%	1 / 1.75%
CD14			1 / 1.28%

Table 4
Overlap of ISGs of same cell subset with different ancestry

cell subsets	# of ISGs in common / overlap rate
CD4	10 / 13.89%
CD8	3 / 4.41%
CD14	13 / 10.57%
CD20	9 / 9.28%