Physical inactivity exacerbates pathologic inflammatory signalling at the single cell level in patients with systemic lupus

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Summary

Background Physical activity is an adjunctive therapy that improves symptoms in people living with systemic lupus erythematosus (SLE), yet the mechanisms underlying this benefit remain unclear.

Methods We carried out a cohort study of 123 patients with SLE enrolled in the California Lupus Epidemiology Study (CLUES). The primary predictor variable was self-reported physical activity, which was measured using a previously validated instrument. We analyzed peripheral blood mononuclear cell (PBMC) single-cell RNA sequencing (scRNA-seq) data available from the cohort. From the scRNA-seq data, we compared immune cell frequencies, cell-specific gene expression, biological signalling pathways, and upstream cytokine activation states between physically active and inactive patients, adjusting for age, sex and race.

Findings We found that physical activity influenced immune cell frequencies, with sedentary patients most notably demonstrating greater CD4+ T cell lymphopenia (P_{adj} = 0.028). Differential gene expression analysis identified a transcriptional signature of physical inactivity across five cell types. In CD4+ and CD8+ T cells, this signature was characterized by 686 and 445 differentially expressed genes (P_{adj} < 0.1). Gene set enrichment analysis demonstrated enrichment of proinflammatory genes in the TNF-α signalling through NF-kB, interferon-γ (IFN-γ), IL2/STAT5, and IL6/JAK/STAT3 signalling pathways. Computational prediction of upstream cytokine activation states suggested CD4+ T cells from physically inactive patients exhibited increased activation of TNF-α, IFN-γ, IL1B, and other proinflammatory cytokines. Network analysis demonstrated interconnectivity of genes driving the proinflammatory state of sedentary patients. Findings were consistent in sensitivity analyses adjusting for corticosteroid treatment and physical function.

Interpretation Taken together, our findings suggest a mechanistic explanation for the observed benefits of physical activity in patients with SLE. Specifically, we find that physical inactivity is associated with altered frequencies and transcriptional profiles of immune cell populations and may exacerbate pathologic inflammatory signalling via CD4+ and CD8+ T cells.

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by pathologic autoantibody formation and immune signalling that can cause inflammation and tissue damage in nearly any organ system. The management of SLE typically involves the use of immunosuppressive medications to control disease activity and prevent flares. Due to the incomplete

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Research in context

Evidence before this study

We searched PubMed on January 12, 2024, for observational studies and clinical trials examining physical activity effects on immune function and clinical outcomes in people with systemic lupus erythematosus (SLE). Study terms included "systemic lupus erythematosus", "physical activity", "gene expression", and "clinical outcomes" with no date or language restrictions. While 51 studies were identified, most examined the effect of physical activity on cardiorespiratory fitness or muscular strength in SLE, and only three included immunologic parameters as outcomes. No studies in SLE have employed bulk or single cell RNA sequencing to comparatively assess transcriptomic markers of systemic inflammation or immune cell frequencies in physically active versus sedentary patients.

Added value of this study

This study demonstrated that amongst SLE patients, physical inactivity is associated with increased cell-specific expression of pro-inflammatory genes implicated in SLE pathogenesis. In CD4+ and CD8+ T cells, this signature was characterized by increased expression of proinflammatory genes, including TNF-α signalling through NF-kB, interferon-γ, IL2/STAT5, and IL6/JAK/STAT3 signalling. Computational modeling of upstream cytokine activation states predicted increased activation of TNF-α, IFN-γ, IL1B, and other proinflammatory cytokines in CD4+ T cells from sedentary patients. Furthermore, we found that physical activity influenced immune cell frequencies, with sedentary patients most notably demonstrating greater CD4+ T cell lymphopenia.

Implications of all the available evidence

We found that people with SLE who are physically inactive have greater CD4+ T cell lymphopenia and upregulation in multiple cell types of SLE-associated genes involved in both innate and adaptive immune signalling compared to those who are more active. These findings provide a probable mechanistic explanation for observations from clinical trials demonstrating improvements in SLE disease-related symptoms following exercise interventions. Taken together, our data suggest that sedentary behaviour exacerbates pathologic immune signalling in SLE, supporting a role for physical activity as an adjunctive treatment approach in the management of this disease.

efficacy and unfavorable side effect profiles of many of these medications, including an elevated risk of serious infections,[1] significant interest exists in adjuvant non-pharmacologic approaches to reduce inflammation and improve symptoms. Avoidance of a sedentary lifestyle is one such approach that has been shown to have multiple benefits in people living with SLE.[2]

Observational studies have found an independent association between physical activity energy expenditure and improvements in pain interference, fatigue, physical function, quality of life, and depressive symptoms among people with SLE.[3–5] Similarly, interventional studies have found significant improvements in fatigue and quality of sleep among SLE patients randomised to physical activity augmentation compared to controls. For example, Gavilan-Carrera and colleagues conducted a 12-week aerobic exercise intervention in SLE and found significantly greater improvements in fatigue in the exercise group compared to the control group.[6] Vordenbaumen et al. found that a combination of physical activity energy expenditure, Mediterranean diet, and not smoking associated with higher quality of life, lower depression, and lower fatigue, with physical activity having the greatest impact on the measured health domains.[5]

A limited but growing body of literature suggests that inflammatory signalling may underlie the harmful effects of a sedentary lifestyle in SLE. For instance, Hashemi and colleagues found that an 8-week exercise program led to lower levels of TNF-α, IL-2, IL-4, and IL-5 in SLE patients.[7] Perandini et al. observed down-regulation of innate and adaptive immune genes (e.g., *TLR3, IFNG, GATA3, FOXP3, STAT4*) following bouts of exercise in patients with SLE.[8] Similarly, Hasni and colleagues found that a 12-week exercise program reduced interferon-stimulated gene expression in a subset of SLE patients.[9]

Despite the growing body of evidence demonstrating important benefits of physical activity among people with SLE, the biological mechanisms underlying these observations remain incompletely understood. Notably, no studies have yet evaluated the relationship between physical activity and inflammatory signalling at the single cell level in people living with lupus. Here we address this gap by leveraging a prospective observational lupus cohort[10] and single-cell RNA sequencing (scRNA-seq) to investigate the impact of a sedentary versus physically active lifestyle on inflammatory gene expression in SLE.

Methods

Study design and participants

We studied subjects enrolled in the California Lupus Epidemiology Study (CLUES), an ongoing prospective longitudinal cohort of adults with SLE. Briefly, starting in 2015, participants for CLUES were recruited through the California Lupus Surveillance Project, which used outpatient, hospital, and laboratory records to identify all SLE patients residing in San Francisco County from 2007 to 2009 (8). Since then, additional participants have been continually enrolled through academic and

community rheumatology clinics in the geographic region. SLE diagnoses were confirmed by study physicians based on (a) ≥4 of the 11 American College of Rheumatology (ACR) revised criteria for the classification of SLE,[11,12] (b) meeting three of the 11 ACR criteria with a documented rheumatologist's diagnosis of SLE, or (c) a confirmed diagnosis of lupus nephritis.

Given our objective to assess the independent association of physical activity with expression of inflammatory genes at the single cell level, participants were included in the current analyses if they had completed a self-reported physical activity assessment and provided a blood sample for scRNA-seq data generation. There were 330 participants in the larger cohort, of whom 123 had the requisite physical activity and scRNA-seq data for the current study.

Ethics

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the institutional review board (IRB) of the University of California San Francisco (IRB number 14-14429). All patients provided written informed consent before enrollment.

Procedures

Following enrollment, patients participated in a research clinic visit, which included a history and physical examination conducted by a rheumatologist with expertise in SLE, collection of peripheral blood, and completion of a structured interview administered by an experienced research assistant. Participants were asked about sociodemographic characteristics, including sex, age, and race. Height and weight were measured during the baseline in-person visit, and body mass index (BMI) was calculated as weight (kg) divided by height (m^2). The presence of depression at baseline was defined by a score ≥10 on the Patient Health Questionnaire (PHQ)-8.[11] Participants were also queried regarding smoking status and major comorbidities such as cardiovascular disease, diabetes mellitus, and cancer. Disease damage was measured with the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index, a physician-assessed measure that provides a composite score of cumulative organ damage in SLE.[12] Disease activity was measured with the Safety of Estrogen in Lupus National Assessment (SELENA) version of the Systemic Lupus Disease Activity Index (SLEDAI), also known as the SELENA-SLEDAI tool.[13] Physical function was measured using the Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Function Scale.[14] Participants were also queried regarding current treatment with glucocorticoids—including dosage and frequency—as well as other immunomodulatory medications.

The primary predictor variable was physical activity, which was measured using the following previously validated[15] single question approach: "Which of the following best describes your physical activity level? a) seldom active; b) moderately active for at least 30 min 3 or more times a week; c) vigorously active for at least 30 min 3 or more times a week." Patients were categorized as physically inactive if they described their physical activity level as "seldom active" (option "a"), and as active if they reported engaging in moderate or vigorous physical activity for at least 30 min on at least three days per week (options "b" and "c").

Single-cell RNA-sequencing (scRNA-seq)

The scRNAseq data was generated using previously published methods.[10] All scRNA-seq analyses were based on an annotated single-cell h5ad object from: https://cellxgene.cziscience.com/collections/436154da-bcf1-4130-9c8b-120ff9a888f2. Briefly, PBMCs were pooled and processed using the 10× Genomics Chromium Single Cell 3′ V2 kit, underwent multiplexed scRNA-seq, and were aligned using the 10× Cell Ranger pipeline. Genome-based de-multiplexing and doublet removal were carried out using freemuxlet[16] and Scrublet.[17] Single cells were clustered by k-nearest neighbor graph and the Louvain algorithm before dimension reduction with Uniform Manifold Approximation and Projection (UMAP).[10] Cell type annotation was performed on each Louvain cluster by comparing their differentially expressed genes and their most highly expressed genes with known cell type markers, using code available on Zenodo at: 10.5281/zenodo.4724043. scRNA-seq analyses were based on an annotated single-cell h5ad object available at: https://cellxgene.cziscience.com/collections/436154da-bcf1-4130-9c8b-120ff9a888f2. The h5ad object was filtered to retain only those from SLE patients for whom physical activity data was available, leaving 195,361 cells from physically inactive patients and 401,751 from physically active patients.

Statistics

All computational and statistical analyses were performed in R v4.3.2. The code is available via Github at: https://github.com/chazlangelier/SLE_PA. We first evaluated differences between key characteristics of participants who met criteria for the present study and the 330 participants enrolled in the full CLUES cohort. Next, we tested for differences in characteristics among participants in the current study based on physical activity status. For all clinical comparisons we used two-tailed t-tests and two-tailed chi-square tests. For clinical data in Table 1, two-tailed P-values were calculated using chi-squared tests for categorical measures and t-tests for continuous measures.

Differential expression analysis

All patients with physical activity and scRNA-seq data (N = 123) were used to assess for differences in cell-

Characteristics	Overall (N = 123)	Physical activity status	Active (N = 81)	P
		Inactive (N = 42)		
Sociodemographic factors:				
Age, mean ± SD	46.1 ± 13.4	44.7 ± 13.4	46.8 ± 13.4	0.420
Female	93.5%	90.5%	95.1%	0.328
Race				0.003
Asian	52.9%	71.4%	43.2%	
White	47.2%	28.6%	56.8%	
SLE specific characteristics:				
Disease activity by SLEDAI, mean ± SD	2.8 ± 2.9	3.0 ± 2.6	2.7 ± 3.0	0.653
Disease damage by SLICC, mean ± SD	1.4 ± 1.8	1.2 ± 1.5	1.6 ± 1.9	0.315
Lupus severity index, mean ± SD	6.5 ± 1.7	6.8 ± 1.4	6.3 ± 1.8	0.135
SLE disease duration, years, mean ± SD	16.5 ± 8.9	12.9 ± 7.6	18.4 ± 9.0	<0.001
History of lupus nephritis	38.2%	42.9%	35.8%	0.445
Current systemic steroid use	45.5%	57.1%	39.5%	0.630
Prednisone ≥7.5 mg/day	20.3%	31.0%	14.8%	0.019
Current hydroxychloroquine use	69.1%	73.8%	66.7%	0.455
Comorbidities and health status[b]:				
Cardiovascular disease	11.5%	14.3%	10.0%	0.480
Hypertension	17.9%	21.4%	16.1%	0.460
Diabetes mellitus, type 2	4.1%	7.1%	2.5%	0.219
History of malignancy	8.2%	4.9%	9.9%	0.342
Depression by PHQ-8 score ≥10	29.3%	35.7%	25.9%	0.258
Body Mass Index				
Obesity	17.1%	19.1%	16.1%	0.675
Current nicotine use	3.3%	2.4%	3.7%	0.711
Physical Function score[c], mean ± SD	49.0 ± 9.3	47.1 ± 9.5	50.0 ± 9.1	0.103

SLEDAI–Systemic Lupus Erythematosus Disease Activity Index, score range 0–105. SLICC–Systemic Lupus International Collaborating Clinics Damage Index. Cardiovascular disease–history of stroke, coronary artery disease, and/or myocardial infarction. Obesity defined as body mass index ≥30 kg/m^2. [a]Values are percent or mean ± standard deviation. P-values calculated using chi-squared tests for categorical measures and t-tests for continuous measures. [b]Comorbidities may have occurred before, after, or concurrent with SLE onset. [c]Assessed by Patient-Reported Outcome Measurement Information System Physical Function (PROMIS PF) 10-item scale.

Table 1: **Characteristics[a] of patients with systemic lupus erythematosus according to physical activity category.**

specific gene expression between physical activity groups. For each cell type in each patient, we prepared pseudobulk gene counts by summing the single-cell gene counts. For example, to calculate the pseudobulk count of *CD3D* in CD4+ T cells in a patient, we sum the counts of *CD3D* genes of all single CD4+ T cells in that patient. Pseudobulk differential expression analysis was performed using the likelihood ratio test (LRT) method in edgeR package (v3.38.4),[18] which performs particularly well for pseudobulk analysis.[19] First, we only kept genes that had at least 10 pseudobulk counts in at least 20% of the patients. Second, we calculated the scaling factors for the library sizes using the calcNormFactor() function and estimated the dispersions using the estimateDisp() function. Third, we performed differential expression testing with the glmFit() and glmLRT() functions from the edgeR package, controlling for patients' age, sex and race as indicated by this R design formula:

$$\text{gene}_i \sim \text{inactive\_status} + \text{age} + \text{sex} + \text{race} \quad (1)$$

where gene_i is the expression of the gene i, inactive_status is the group identity of an individual (physically active or physically inactive), age is the continuous variable of their biological age in years, sex is their biological sex (male or female), and race is their race (Asian or White). The glmFit() function fits a negative binomial generalized log-linear model to the pseudobulk counts of each gene, and models the mean-variance relationship of the gene counts with a quadratic equation. We confirmed our data fit the model assumptions and that pseudobulk gene counts followed a negative binomial distribution and that the mean-variance relationship was quadratic. The glmLRT() functions calculates the P-value on the variable of interest (inactive_status variable) using the likelihood ratio test. Finally, we adjusted the differential expression P-values using the Benjamini-Hochberg correction and considered an adjusted P-value of <0.1 significant.

Pathway enrichment analyses

The analysis of Hallmark pathway enrichment was performed using the gene set enrichment analysis tool in the clusterProfiler package (v4.4.4).[20] The Hallmark pathway-associated genes were taken from the msigdbr package (v7.5.1). The genes were pre-ranked using the following metric:

$$-\left(\log_{10} Pvalue\right) \times sign\left(\log_2 FC\right) \quad (2)$$

where P-value is the unadjusted differential expression P-values, *sign* is the sign function, and FC is the differential expression fold change (a positive $\log_2 FC$ indicates that a gene is upregulated in inactive patients compared to active patients).

All genes were used as input to the GSEA function. The gene-concept network of top Hallmark pathways was generated using the cnetplot() function from the enrichplot package (v1.16.2). Ingenuity Pathway Analysis (IPA)[21] was carried out on differentially expressed genes with a Benjamini-Hochberg adjusted P-value <0.1 ranked by log2 fold change. Significant IPA results were defined as those with a Z-score absolute value greater than 2 or an overlap P-value <0.05.

Sensitivity analyses

We also conducted two sensitivity analyses. In the first sensitivity analysis, to address the possibility of confounding by treatment with steroids, we included a covariate for taking moderate or high doses of steroids (defined by a steroid dose equivalent to at least 7.5 mg/day of prednisone) in the differential expression model. More specifically, we used the following R design formula:

$gene_i \sim inactive\_status + age + sex + race + steroid$ (3)

where steroid indicates whether the individual was taking ≥7.5 mg/day prednisone (yes or no).

In the second sensitivity analysis, to address the possibility of confounding from inability to participate in physical activity among patients with more severe or active disease, we adjusted for physical function in our R design formula:

$gene_i \sim inactive\_status + age + sex + race + phys\_funt$ (4)

where phys_funt is a continuous measurement of physical function.

Role of funders

The funders were not involved in the writing of the manuscript or decision to submit for publication.

Results

Patient cohort

We studied 123 patients with SLE prospectively enrolled in the California Lupus Epidemiology Study (CLUES), who had both physical activity data and PBMC scRNA-seq data[10] collected (Fig. 1). Based on the patients' report of their physical activity behaviour (described in Methods), 81 physically active and 42 sedentary patients were identified. The physically inactive group had greater representation among Asian versus White patients, were more likely to be receiving ≥7.5 mg per day of prednisone, and had a shorter SLE disease duration (Table 1). No differences in disease activity (SLEDAI), disease damage (SLICC), disease severity (lupus severity index), or comorbidities existed between the physical activity groups (Table 1). When we compared key clinical characteristics between CLUES participants who were and were not included in the present study (Supplemental Table S1), we found that the two groups were largely similar. The only statistically significant differences between CLUES patients in the present study versus those who were not eligible were that the latter group had marginally higher means for disease damage and body mass index.

Cell populations identified by scRNA-seq

From the single-cell gene expression data of 597,112 PBMCs, 11 immune cell types were identified and visualized by uniform manifold approximation and projection (UMAP) (Fig. 2A). In both the physically inactive and active groups, CD14+ classical and CD16+ nonclassical monocytes (cM and ncM) were identified; as were conventional and plasmacytoid dendritic cells (cDC and pDC); CD4+ and CD8+ T cells; natural killer cells (NK); B cells (B); plasmablasts (PB); proliferating T and NK cells (Prolif); and progenitor cells (Progen) (Fig. 2B). Evaluation of cell type frequencies demonstrated differences between activity groups, with higher proportions of CD4+ T cells and NK cells in the physically active patients (Fig. 2C, Supplemental Figure S1A). We further analyzed the abundance of different lymphoid subpopulations (Fig. 2D), and observed that

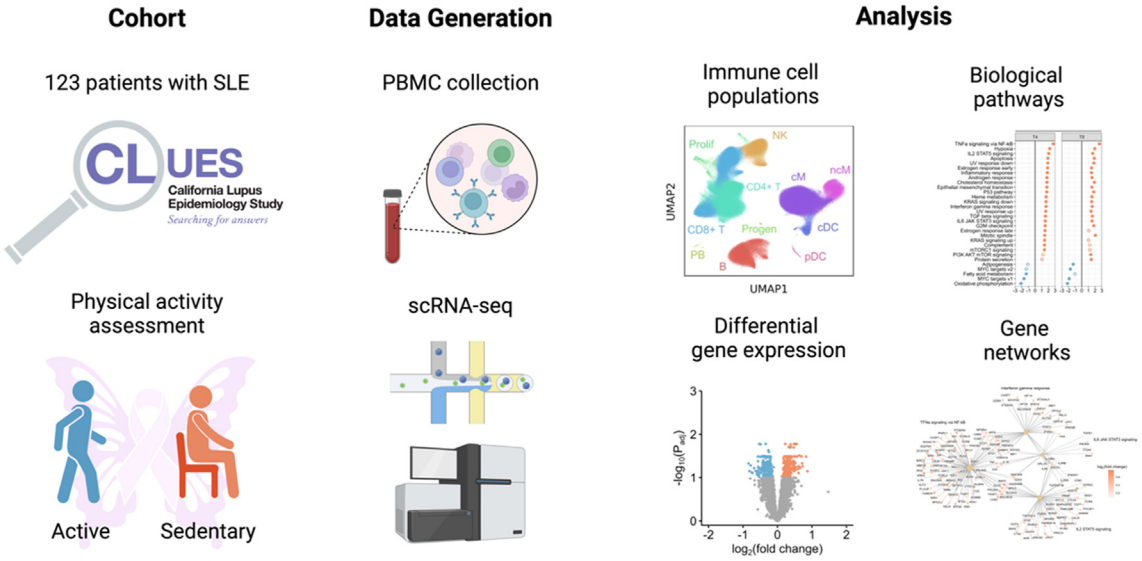

***Fig. 1:* Study overview.** Patients with systemic lupus erythematosus (SLE) enrolled in the California Lupus Epidemiology Study (CLUES) were compared based on physical activity status (active versus sedentary). Single cell RNA sequencing of PBMCs was carried out to identify and profile immune cell populations. Cell frequencies, gene expression, biological pathways, and gene networks were compared between physically active and sedentary groups.

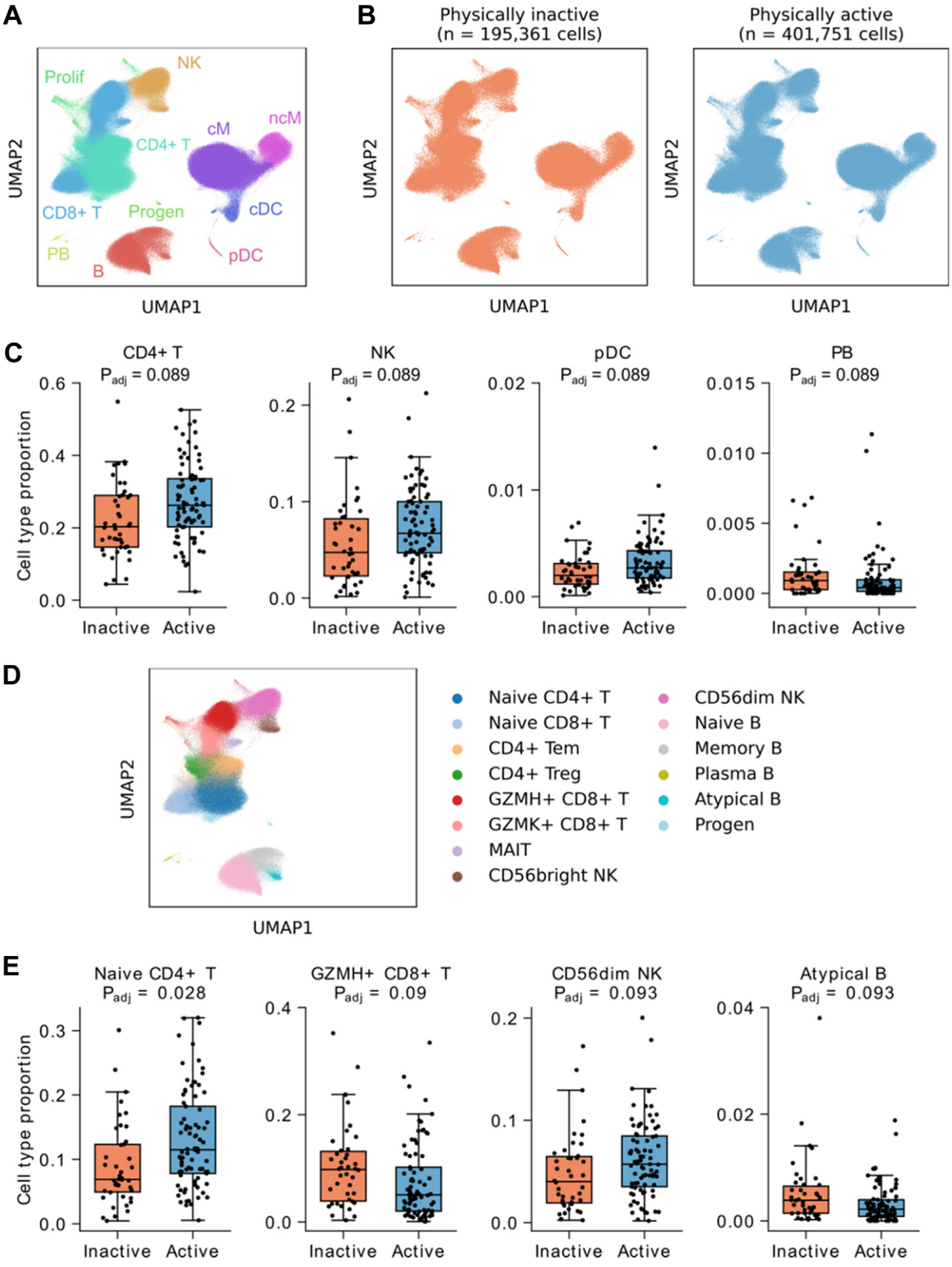

***Fig. 2*: scRNA-seq identifies differences in immune cell frequencies and gene expression based on physical inactivity**. **(A)** UMAP plot of all single cells used in the study, coloured by the cell types. There are 11 cell types: CD4+ T cells and CD8+ T cells, B cells, classical and nonclassical monocytes (cM and ncM), natural killer cells (NK), plasmablasts (PB), conventional and plasmacytoid dendritic cells (cDC and pDC), proliferating lymphocytes (Prolif), and CD34 progenitors (Progen).[10] **(B)** UMAP plots of single cells from physically inactive (left) and physically active (right)

physical inactivity was associated with fewer naïve CD4+ T cells, and more GZMH+ CD8+ T cells (Fig. 2E, Supplemental Figure S1B).

Cell-specific transcriptomic differences based on physical inactivity

We next asked whether gene expression differed based on physical activity group across each of the cell types, adjusting for age, sex and race. Differentially expressed genes were identified in five of the cell types, with the strongest signals found in CD4+ T cells (686 genes at false discovery rate (FDR) < 0.1) and CD8+ T cells (445 genes) (Fig. 3A and B, Supplemental Data 1). Several proinflammatory genes implicated in SLE pathogenesis were upregulated in CD4+ and CD8+ T cells among the physically inactive patients, including *IRF1*[22], *CD69*[23], *IL1B*.[24]

Gene set enrichment analysis (GSEA) further clarified the biological relevance of these results and demonstrated that physical inactivity was associated with upregulation of several inflammatory pathways directly implicated in the immunopathology of SLE. In both CD4+ and CD8+ T-cells, inactivity correlated with higher TNF-α, IL2/STAT5, interferon-γ (IFN-γ) and IL6/JAK/STAT3 signalling (Fig. 3C, Supplemental Data 2). Computational prediction of upstream cytokine activation states from transcriptomic data corroborated GSEA results and indicated higher activation of several canonical cytokines in CD4+ T-cells, including TNF-α, IL1A/B, IFN-γ, IL2, IL17A, and others (Fig. 3D). In CD8+ T-cells, this approach predicted greater activation of TNF-α and IL5 in the sedentary group compared to the rest of the patients (Fig. 3E).

Although classical monocytes and B cells showed fewer differentially expressed genes compared to CD4+ and CD8+ T cells (Fig. 3A), we asked whether the former two cell types displayed any differences at the biological pathway level. Overall, we observed similar results, with TNF-α signalling and IL2/STAT5 signalling pathways being upregulated in physically inactive patients (Supplemental Figure S2).

To further investigate relationships between genes and pathways influenced by physical activity in SLE patients, we constructed a gene concept network plot. We focused on four key proinflammatory pathways associated with SLE pathogenesis upregulated in sedentary patients: TNF-α signalling via NF-kB, IFN-g response, IL6/JAK/STAT3, and IL2/STAT5 signalling (Fig. 4). Cross pathway connections were observed for several genes, highlighting the pleiotropic effects of physical inactivity-associated genes on inflammatory signalling pathways in SLE. For instance, increased *IRF1* expression contributed to upregulation of several pathways including interferon gamma signalling, IL6/JAK/STAT3 signalling, and TNF-α signalling through NF-kB.

We considered whether our results might be influenced by treatment with immunosuppressive medications, and specifically by treatment with the oral steroid equivalent of at least 7.5 mg per day of prednisone. Even though a higher proportion of physically inactive patients received this dose of steroids (Table 1), which would be expected to bias our results towards the null, we conducted a sensitivity analysis in which we included steroid treatment as a covariate in the differential expression and gene set enrichment analyses. We found that the TNF-α signalling via NF-kB and IL2/STAT5 signalling pathways remained significantly upregulated in CD4+ T cells and CD8+ T cells among physically inactive patients compared to active patients (Supplemental Figure S3, Supplemental Data 3), indicating that steroid use did not significantly change the primary findings of our analyses.

Lastly, we considered that differences in the ability to engage in exercise among patients with more active disease might explain our findings. To test for this possibility, we conducted a second sensitivity analysis in which physical function—defined as an individual's ability to perform both basic and instrumental activities of daily living[25]—was included as a covariate. Though physical function and physical activity are related, physical function represents the ability to do certain activities whereas physical activity represents the extent to which an individual does them. In this second sensitivity analysis, GSEA demonstrated that pathway results remained consistent with those from the main analysis for both CD4+ T cells and CD8+ T cells (Supplemental Figure S4, Supplemental Data 4). Specifically, even after adjusting for physical function, we continued to see upregulation of interferon and other proinflammatory signalling pathways among the physically inactive patients relative to the rest of the patients in the cohort.

Discussion

Nonpharmacologic therapies such as physical activity can complement standard medication-based SLE treatments, mitigate the risk of cardiovascular disease and other important comorbidities, and offer the benefit of conferring less side effects. While both observational studies and randomised controlled trials suggest that

patients. (**C**) Box plots showing the proportion of the 4 significant cell types in the physically active (n = 81) and inactive (n = 42) groups (P-values, two-sided Mann–Whitney test with Benjamini-Hochberg correction). (**D**) UMAP plot showing the lymphoid subpopulations: CD4+ and CD8+ naïve T cells, CD4+ effector memory and regulatory T cells, GZMH+ and GZMK+ CD8+ T cells, mucosal-associated invariant CD8+ T cells (MAIT), $CD56^{bright}$ and $CD56^{dim}$ natural killer cells. (**E**) Box plots showing the proportion of the 4 significant lymphoid subtypes in the physically active (n = 81) and inactive (n = 42) groups. Boxes indicate the first and third quartiles, middle lines indicate the median value, and whiskers extend to 1.5 × the interquartile range below the first quartile and above the third quartile.

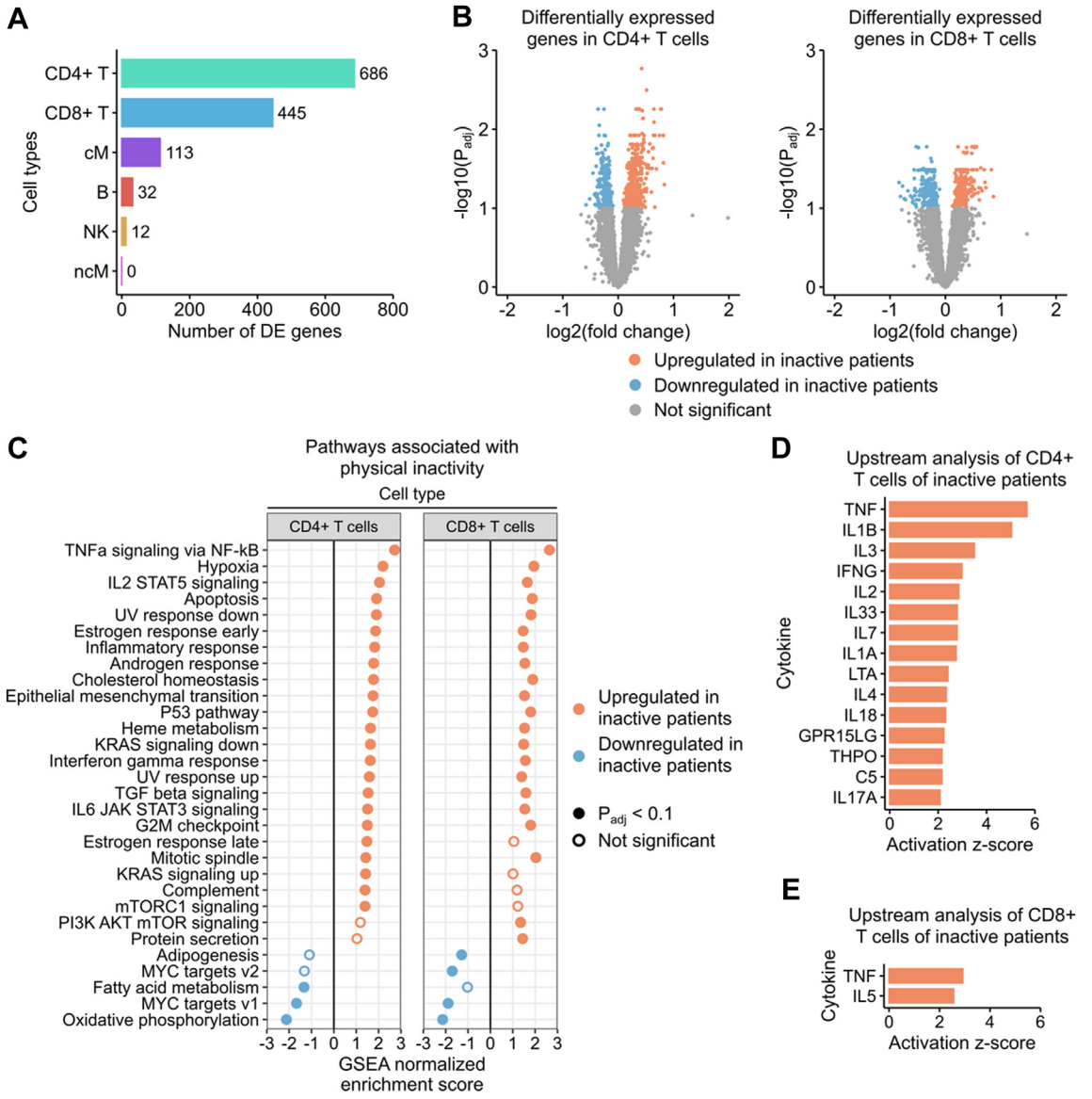

***Fig. 3:* Physical inactivity drives proinflammatory gene expression in T cells**. (**A**) Bar plot showing the number of differentially expressed (DE) genes between the physically active (n = 81) and inactive (n = 42) groups at an adjusted P-value (Padj) < 0.1 for each of the 6 most abundant cell types. Data regarding more finely resolved cell subtypes are presented in Supplemental Figure S1. (**B**) Volcano plots of differential expression analysis in CD4+ T cells and CD8+ T cells. There were 686 and 445 DE genes (FDR <0.1) in CD4+ T cells and CD8+ T cells, respectively. A positive $\log_2$(fold change) indicates that a gene is upregulated in physically inactive patients compared to active patients. (**C**) Dot plots showing Hallmark pathways that are statistically significantly associated with physical inactivity in CD4+ T cells and CD8+ T cells (FDR <0.1). (**D, E**) Bar plots showing the cytokines predicted by Ingenuity Pathway Analysis to be activated in (**D**) CD4+ T cells and (**E**) CD8+ T cells of physically inactive patients compared to active patients.

physical activity improves patient reported outcomes[2] in SLE, the mechanisms underlying these benefits have remained in question. Leveraging PBMC single cell transcriptomics from a prospective cohort, we evaluated the impact of physical activity on circulating immune cells and inflammatory gene expression. We found that, in our lupus cohort, physical inactivity independently associates with both the frequencies and transcriptional profiles of immune cell populations. Importantly, we also found that sedentary behaviour independently associates with pathologic inflammatory signalling in both CD4+ and CD8+ T cells in SLE.

Several canonical inflammatory pathways implicated in SLE pathogenesis were upregulated in T cells from sedentary individuals. These include IFN-γ signalling, which regulates several immune cells, including B-cells

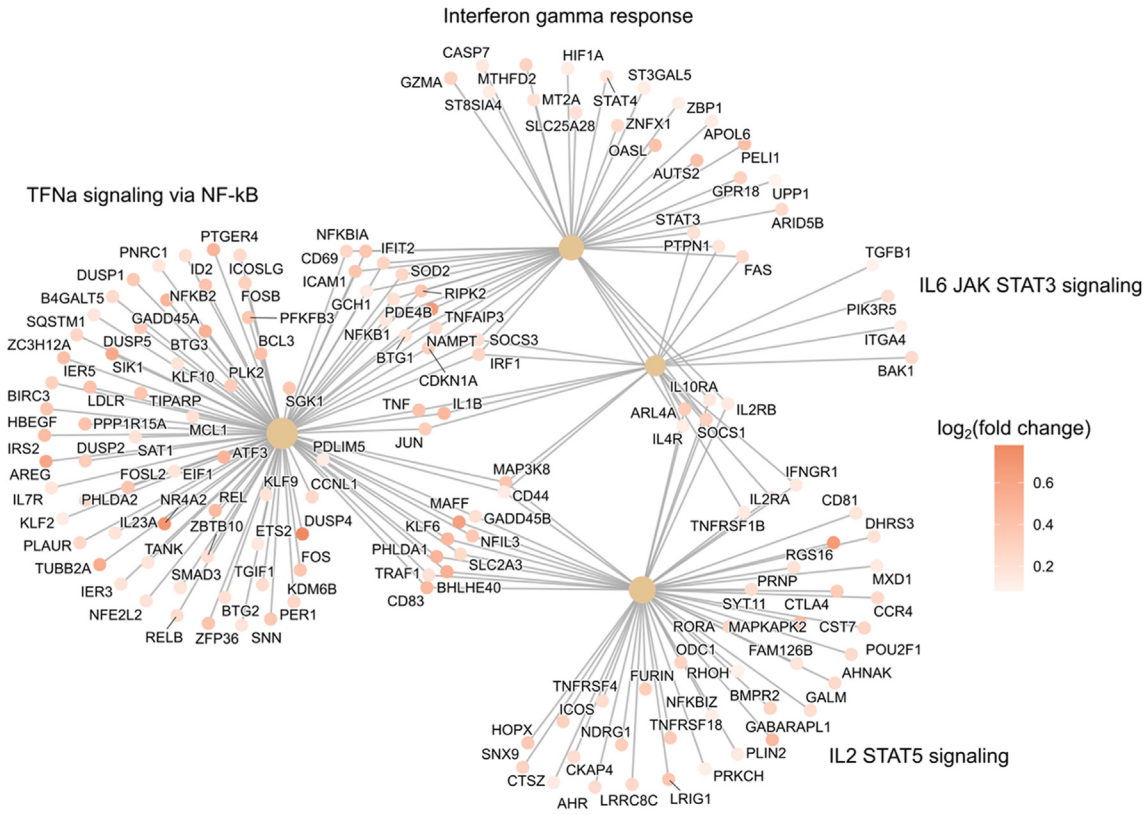

Fig. 4: **A gene network drives proinflammatory signalling in CD4+ T cells**. A gene-concept network plot of 4 immune-related Hallmark pathways in CD4+ T cells (TNF-α signalling via NF-kB, IFN-γ response, IL6 JAK STAT3 signalling, and IL2 STAT5 signalling pathways). The gene dots are coloured by the genes' log_2(fold change), and a positive and negative log_2(fold change) indicate that the gene is upregulated and downregulated in physically inactive patients, respectively.

responsible for the auto-antibody production central to SLE pathogenesis.[26] Elevated IFN-γ activity is a feature of severe SLE[27] complicated by nephritis and arthritis, and rising levels of this cytokine have been shown to precede auto-antibody detection among people who go on to develop SLE.[26,28]

TNF-α signalling through NF-kB was another pathway upregulated in T cells from sedentary SLE patients. TNF-α contributes to SLE immunopathogenesis via multiple mechanisms, including by acting as a B-cell growth factor and by augmenting autoantibody production.[29] Other genes in the NF-KB pathway that were upregulated among sedentary versus active lupus patients included *CD69* and *IL1B*. *CD69* is relevant to SLE as it is known to intensify autoreactive T cell activity in SLE.[23] *IL1B* is also relevant to the pathophysiology of SLE given that mice lacking *IL1B* are resistant to induction of experimental SLE.[30]

Lymphopenia is an established biomarker of SLE and recent work has suggested a role for CD4+ T cell deficiency in disease pathogenesis.[10] We found that sedentary individuals had significantly lower proportions of CD4+ T-cells, suggesting a potential role for physical activity in offsetting the T lymphopenia of SLE. Indeed, other studies have demonstrated that physical activity increases CD4+ T-cell populations.[31]

The most consistent finding related to physical activity effects in SLE within the existing literature is the association between more time in moderately intense physical activities and lower levels of fatigue. For example, three separate randomised controlled trials and one uncontrolled intervention[9] testing the impact of exercise training on clinical outcomes in SLE found that aerobic exercise significantly decreased fatigue.[6,32,33] The impact of physical activity on SLE disease activity is less well studied, however three small, likely underpowered, exercise RCTs that measured SLEDAI found no significant difference in disease activity among lupus patients randomised to exercise versus control.[34–36] These prior studies are consistent with our observation that there was no significant association between physical activity and SLE disease activity (via SLEDAI). This finding of similar SLEDAI scores despite significant differences in immune signalling between physical activity groups suggests that either the SLEDAI score is insufficiently sensitive to reflect the cell specific transcriptional differences we observed, or that the immune signalling differences we detected are more relevant to other

clinical outcomes not captured in the SLEDAI score, such as fatigue. To address this outstanding question, there is an important need for a future, appropriately powered, clinical trial to test the effects of physical activity on clinical outcomes as well as biological markers of inflammatory signalling in SLE.

Only a few prior studies—all with small sample sizes ranging from 8 to 16 participants—have examined the effects of physical activity in SLE patients at the molecular level. In line with our findings, Hasni and colleagues found that a subset of women with SLE who completed an exercise training program had significant reductions in the expression of interferon stimulated genes.[9] Hashemi and colleagues evaluated the effect of an eight-week exercise program on serum cytokine levels and found that TNF-α, IL2, IL-4, and IL-5 decreased significantly in the intervention as compared with the control group.[7] Similarly, we found that more physically active patients had greater TNF-α and IL2/STAT5 signalling in CD4+ and CD8+ T cells compared to those who were sedentary. A third study by Perandini and colleagues also found evidence for decreased signalling via TNF-α following exercise training, as there was lowing resting soluble TNF receptor at the end of the study among SLE patients randomised to the training program.[37] Taken together, our study builds on prior research demonstrating an attenuating effect of physical activity on inflammatory signalling in people living with lupus.

The anti-inflammatory effects of an active lifestyle may extend beyond SLE and be generalisable to other autoimmune diseases such as rheumatoid arthritis (RA). In a study by Bartlett and colleagues that examined the effects of a 10-week high-intensity interval walking program for people with RA, there was a significant improvement in joint swelling, disease activity, and erythrocyte sedimentation rate following the exercise intervention.[38] Furthermore, in a recent whole blood transcriptional profiling study of rheumatoid arthritis patients, moderate physical activity was associated with downregulation of pathologic inflammatory signalling pathways such as IL17, TNF-α and interferon signalling.[39]

The primary limitation of this study is the observational design, which comes with the risk of unmeasured confounders and precludes the ability to make strong statements about causation between variables. We hypothesise that physical inactivity exacerbates pathogenic T cell inflammatory signalling in people with SLE, but we acknowledge that the relationship between physical activity and immune function is likely bidirectional since people with more active lupus have more pathogenic inflammation and less ability to maintain a physically active lifestyle. However, we addressed potential confounding from inability to engage in physical activity among participants with more active lupus by controlling for physical function in a sensitivity analysis, and we found stable results compared to the main analysis. Another limitation of this study is that we did not have quantitative assessments of physical activity (e.g., actigraphy), and thus could not examine the impact of different levels of activity engagement on gene expression. Finally, though we controlled for several potentially confounding factors—including immunosuppressive treatment and ability to engage in physical activity—there remains the risk of confounding by other factors not measured.

The limitations of this study are outweighed by several strengths. First, we examined a novel and clinically relevant question using one of the most extensive scRNAseq datasets available from individuals with SLE. In addition, we have carried out the largest study to date examining the relationship between physical activity and inflammatory signalling in patients with autoimmune disease. Finally, we leveraged a large prospective observational cohort with detailed clinical phenotyping and rigorous, physician-confirmed diagnoses of SLE.

In summary, we found that physical inactivity may exacerbate CD4+ T cell lymphopenia and upregulate T-cell inflammatory signalling pathways implicated in SLE pathogenesis. These findings provide the first mechanistic evidence for a disease-modifying effect of physical activity in SLE. Our findings also underscore the importance of public health programs to support engagement in physical activity among this high-risk population.

Contributors

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All authors read and approved the final version of the manuscript.

Data sharing statement

Annotated scRNA-seq data are available at: https://cellxgene.cziscience.com/collections/436154da-bcf1-4130-9c8b-120ff9a888f2.

Code sharing

All code is available via Github at: https://github.com/chazlangelier/SLE_PA.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105432.

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