Research Paper

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Unbiased immune profiling reveals a natural killer cell-peripheral nerve axis in fibromyalgia

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Abstract

The pathophysiology of fibromyalgia syndrome (FMS) remains elusive, leading to a lack of objective diagnostic criteria and targeted treatment. We globally evaluated immune system changes in FMS by conducting multiparametric flow cytometry analyses of peripheral blood mononuclear cells and identified a natural killer (NK) cell decrease in patients with FMS. Circulating NK cells in FMS were exhausted yet activated, evidenced by lower surface expression of CD16, CD96, and CD226 and more CD107a and TIGIT. These NK cells were hyperresponsive, with increased CCL4 production and expression of CD107a when co-cultured with human leukocyte antigen null target cells. Genetic and transcriptomic pathway analyses identified significant enrichment of cell activation pathways in FMS driven by NK cells. Skin biopsies showed increased expression of NK activation ligand, unique long 16–binding protein, on subepidermal nerves of patients FMS and the presence of NK cells near peripheral nerves. Collectively, our results suggest that chronic activation and redistribution of circulating NK cells to the peripheral nerves contribute to the immunopathology associated with FMS.

Keywords: Fibromyalgia, Chronic pain, Widespread pain, Peripheral nerves, Small fiber neuropathy, ULBP, NKG2D, Natural killer cells, Flow cytometry, Skin, Whole blood, RNA, DNA

1. Introduction

Fibromyalgia syndrome (FMS) is a common chronic primary pain condition[18] with global prevalence ranging from 1% to 12.5% in women and 0% to 5.1% in men.[20,73] Along with chronic widespread pain, this syndrome is characterized by presence of fatigue, sleep disturbance, depression, and cognitive difficulties. This syndrome is often associated with an array of other symptoms such as restless legs syndrome, irritable bowel syndrome, vulvodynia, irritable bladder, xerostomia, and dry eyes.[4] The diagnosis of FMS lacks objective biomarkers or reliable and valid clinical measures.[30,87] Because the diagnostic criteria are based on clinical assessment and not the pathophysiology of FMS, this often leads to misdiagnosis and contributes to heterogeneity among the patient group. Although therapeutic strategies including physiotherapy, cognitive behavioral therapy, antidepressants, and neuropathic pain medications may have some efficacy in reducing pain and improving quality of life, the magnitude of these improvements is often not clinically meaningful to the patients.[64] Finally, incomplete understanding of the etiology and pathogenesis of FMS is a major obstacle in modeling

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the disease in animal assays, further hampering preclinical research. In summary, the lack of objective diagnostic markers and poorly understood aetiopathogenesis of FMS are the 2 most important obstacles in diagnosing, treating, and researching FMS.

Both central and peripheral nervous systems have been shown to be affected in FMS. Changes in gray matter volume in specific regions of the brain, decreased functional connectivity in the descending pain-modulating system, and increased activity in the pain matrix are suggestive of central sensitization in FMS.[14,83,90] Increased peripheral nerve demyelination,[15] decreased dermal and intraepidermal nerve fibers in patients with FMS,[16,28,29] and the presence of sensory symptoms[46] such as paraesthesia, hyperalgesia, and allodynia are suggestive of peripheral nerve involvement in FMS.

Epidemiological studies have shown that allergic[6,7,82] and autoimmune[19,32] comorbidities are common among patients with FMS. Targeted exome sequencing[91] and proteome-wide[37] studies have identified molecular signatures consistent with low-grade chronic inflammation in patients with FMS. Transcriptome-wide approaches found the relevance of immune response pathways and homeostasis in FMS.[59] Previous studies also suggest a defective cytotoxic immune response,[8] increased activation of lymphocytes, and increased expression of cell adhesion molecules on neutrophils and monocytes[60] in FMS. However, specific immune players and neuroimmune interactions involved in the pathophysiology of FMS remain to be discovered.[77] To further our understanding of the immune system's involvement in FMS, here, we aimed to identify immunophenotypic differences between individuals with and without FMS in a hypothesis-free manner.

2. Methods

2.1. The Canadian cohort

2.1.1. Study population

The sample size was based on a previous study[76] and was calculated using a power and sample size calculation tool.[26] Forty-four patients with FMS and 46 matched controls were deemed sufficient to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power, $1 - \beta$) 80% and type I error (α) $\leq$ 5%. The Canadian cohort of patients with FMS and controls was collected under the research project approved by the Institutional Review Board, Faculty of Medicine, McGill University (Institutional Review Board Study Number A05-M50-14B). Individuals with a clinician-rendered diagnosis of FMS based on the 2010 American College of Rheumatology diagnostic criteria[88] attending the rheumatology clinic or the Alan Edwards Pain Management Unit of the Montreal General Hospital (McGill University Health Centre, MUHC) were invited to participate. Participants with FMS were also recruited by advertising our study among members of the Association de Fibromyalgie du Quebec. In addition, recruitment of both participants with FMS and controls included word of mouth, advertising the study in local newspapers, at MUHC and McGill University bulletin boards and web sites, and through mass emails.

The following inclusion criteria were applied to determine eligibility to participate in this study: the ability to write and speak in English or French, provide signed and dated informed consent, and aged 40 years and older. Individuals were excluded from the study if they had other diagnosed pain conditions that could explain their pain, a medical or psychiatric condition that was uncontrolled, or previous or current drug or alcohol abuse. Finally, in control participants, FMS diagnosis was excluded by medical examination by the clinician using the 2010 diagnostic criteria.[88] In addition, control participants must never have been diagnosed with any chronic pain condition or have a history of depression.

2.1.2. Outcome measures

The primary outcome measure for this study was the case status for FMS. Apart from age, sex, body mass index (BMI), and detailed medical history, other outcome measures collected are summarized in Supplementary Table 1 (available at http://links.lww.com/PAIN/B515). All demographic, anthropometric, clinical, and patient-reported outcomes were collected and managed using the Research Electronic Data Capture tool,[38] which is a secure, web-based application designed to support data capture for research studies hosted at the Research Institute of the McGill University Health Centre.

2.1.3. Blood sample collection

For the described study, 15 mL of peripheral blood was collected in ethylenediaminetetraacetic acid vacutainer tubes from each participant from the median cubital vein. Two and a half milliliter of whole blood was preserved in a PAXgene Blood RNA Kit (762174; PreAnalytiX, Hombrechtikon, Switzerland) and stored at −80°C for RNA extraction. Peripheral blood mononuclear cells (PBMCs) were fractionated from the remaining whole blood using a Ficoll-Paque PLUS (17144002; GE Healthcare Life Sciences, Mississauga, Canada)-based density gradient and SepMate-50 tubes (85450; STEMCELL Technologies , Vancouver, Canada).[81] PBMCs (3 vials with 5 million cells in each) were resuspended in heat-inactivated human plasma–derived serum (H4522; Sigma-Aldrich, Oakville, Canada) and 10% dimethyl sulfoxide (D128-500; Fisher Scientific, Mississauga, Canada) and cryopreserved in liquid nitrogen for flow cytometry and natural killer (NK) activation assays.

2.1.4. mRNA sequencing

RNA from whole blood was isolated from PAXgene collection tubes according to the provider's protocol (PreAnalytiX GmbH, 08/2005, REF: 762174). The average mRNA concentration was 120 ng/μL, and 25 μL of mRNA per sample was sequenced using Illumina's NovaSeq 6000 S2 SR100 platform at Génome Québec, Montreal, QC, Canada.

2.1.5. Flow cytometry

One vial of the cryopreserved PBMCs was split into 3 to stain for cytokine production ("cyto panel"), chemokine receptor expression ("chemo panel"), and regulatory T cells or Tregs ("Treg panel"). Another vial was split into 2 to stain for B cells, monocytes, and dendritic cells or DCs ("BMD panel") and NK cells ("NK panel"). Experimental design, selection of the antibodies and fluorochromes, antibody concentrations, and flow cytometer photomultiplier tubes' voltages were optimized to minimize technical artifacts in downstream computational analysis.[11] Details of the excitation lasers, channel filters, clones, antibody isotypes, fluorochromes, manufacturer and catalog numbers, and the titer of reagents used for all the flow cytometry panels are summarized in Supplementary Table 2 (available at http://links.lww.com/PAIN/B515). Cryopreserved PBMCs were rapidly thawed in a 37°C water bath, resuspended in Iscove's

Modified Dulbecco's Media with 10% fetal bovine serum, and incubated at 37°C in a 5% CO_2 incubator for 1 hour to improve detection of cell surface markers.[54] Viability staining was performed using Fixable Viability Dye eFluor 780 (65-0865-14; Thermo Fisher Scientific, Waltham, MA) and Zombie NIR (423106; BioLegend, San Diego, CA), and nonspecific antibody binding was minimized by the use of TruStain FcX reagent (422302; BioLegend, San Diego, CA), as per the manufacturer's instructions. Subsequently, the cells were stained for extracellular markers. Intracellular staining was preceded by fixation with 2% paraformaldehyde and permeabilization as per the manufacturer's protocol (421002; BioLegend, San Diego, CA). After the staining and fixation, the PBMCs were stored at 4°C in the dark for data acquisition on the subsequent day. Unstained cells, single-stained cells, and fluorescence minus one staining were used as controls. UltraComp eBeads were used for compensation of all fluorochromes. Using SPHERO alignment particles, the coefficients of variation, peak channels, and histogram distributions were aligned before every flow cytometry run to standardize data acquisition across all the batches. Around 2 million total events were acquired from each sample on an LSRFortessa instrument (BD Biosciences, Mississauga, ON, Canada) at the McGill University's flow cytometry and cell sorting facility and the immunophenotyping platform of Research Institute of the McGill University Health Centre. Acquisitions of all the batches were performed within 24 hours of staining.

2.1.6. Natural killer activation assay

One vial (5 million cells) of cryopreserved PBMCs from randomly chosen 14 cases and 14 controls was thawed, counted, and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 IU/mL penicillin, and 50 mg/mL streptomycin (henceforth, R10 medium) at 4×10^6/mL for overnight resting. Each sample was split into 4: 2 were stimulated, or not (as a negative control), by coculture with human leukocyte antigen null ($HLA^{-/-}$) cells and 2 were used in an antibody-dependent NK activation (ADNKA) assay.

The $HLA^{-/-}$ assay is described in detail elsewhere.[80] For this assay, lymphoblastoid cell line 721.221, a kind gift from Dr. Galit Alter (Ragon Institute, Harvard University, Cambridge, MA) to Dr. Nicole Bernard, was used to stimulate NK cells. In short, rested PBMCs (effector) were cocultured with 221 cells (target) at an E:T ratio of 5:1 in 200 μL of R10 in V-bottom 96-well plates. PBMCs cultured alone in R10 served as an unstimulated negative control.

Details of the ADNKA assay are described elsewhere.[27,56] CEM.NKR-CCR5 (CEM) cells (from the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, Germantown, MD, from Dr. Alexandra Trkola) infected with the viral isolate NL4-3–Bal–IRES–HSA were sorted for infected CEM cells.[27,39] HIV+Ig (pooled plasma from HIV-infected donors) (catalog #3957; HIV−Ig from NABI and NHLBI) obtained from the NIH AIDS Reagent Program or HIV−Ig served as the opsonizing antibodies. Infected CEM cell count of 1×10^6 cells (50 μL) and 50 μL of prediluted opsonizing antibodies (HIV+Ig or HIV−Ig, 50 μg/mL in R10) were added to the rested PBMCs (effector) in V-bottom 96-well plates (E:T ratio of 1:1).

One hour after the initiation of the cocultures, GolgiStop (cat. 554724; BD Biosciences, Mississauga, Canada.) and GolgiPlug (cat. 555029; BD Biosciences, Mississauga, Canada.) were added. BV711-conjugated anti-CD107a (5 μL/well; cat. 328640; BioLegend, San Diego, CA) was also added at the beginning of the coculture. Both the cocultures ($HLA^{-/-}$ and ADNKA) were incubated for 6 hours at 37°C in a humidified 5% CO_2 incubator. After incubation, the plates were wrapped in aluminum foil and stored in a 4°C fridge overnight. The next day, the cells were stained with UV Live/Dead Fixable Blue cell stain kit, as per manufacturer's directions (cat. L34961; Thermofisher, Waltham, MA) and with reagents described in Supplementary Table 2 (available at http://links.lww.com/PAIN/B515) (NKA panel).

2.2. The German cohort

The second cohort of patients with FMS were enrolled in the research project approved by the Ethics Committee of the University of Würzburg, Medical Faculty, Germany (#121/14), and all study participants gave written informed consent. The details of the study population and following skin biopsy procedures are summarized elsewhere.[29] In short, 382 participants were screened and 117 women (median age of 52 years, range: 22-75 years) with FMS were enrolled at the Department of Neurology, University of Würzburg, Germany. Inclusion criteria were age ≥18 years and a diagnosis of FMS made by a neurologist according to the most current diagnostic criteria at the time.[29] The Fibromyalgia Survey Questionnaire (FSQ) that assesses the key symptoms of FMS was also administrated, and FSQ severity score was calculated.

2.2.1. Skin microscopy

Skin punch biopsies measuring 6 mm were obtained from the right lateral lower leg and upper thigh of the German cohort participants. The skin punch biopsies obtained from the lower legs of 17 patients with FMS and 11 controls were randomly selected for this study. These samples were processed to determine the intraepidermal nerve fiber density as previously described.[29] In brief, 40-μm cryosections were immunoreacted with rabbit anti-human antibodies against the pan-axonal marker protein gene product (PGP) 9.5 (1:1000; Zytomed, Berlin, Germany, 516-3344) and a donkey anti-rabbit Cy3 coupled secondary antibody (1:100; Dianova, Hamburg, Germany, 711-545-152). Intraepidermal nerve fiber density was determined following standardized counting rules[52] by an investigator blinded to subject allocation on an Axiophot microscope (Zeiss, Oberkochen, Germany) equipped with an AxioCam MRm camera (Zeiss, Oberkochen, Germany) and SPOT software (Diagnostic Instruments, Sterling Heights, MI). Intraepidermal nerve fiber density was defined as normal if there were ≥ 5.4 fibers/mm according to the normative values from the skin biopsy laboratory of Würzburg University.

For the investigation of unique long 16–binding protein (ULBP, ligand for the CD314 or NK group 2D [NKG2D] receptor) and NK cells, 20-μm sections were prepared and 2 sections were loaded per slide. The slides were blocked with 10% bovine serum albumin in phosphate-buffered saline (PBS) for 30 minutes at room temperature. Double-labeling was performed using anti-ULBP goat polyclonal antibody (Ab,1:50; R&D 1298) as a marker for NK cell receptor, PGP9.5 rabbit anti-human Ab (1:200; Zytomed, Berlin, Germany, 516-3344) as an axonal marker, anti-CD56 goat monoclonal Ab (mAb, 1:100; R&D, Minneapolis, MN AF2408) as a marker for NK cells, and CD3 rat anti-human mAb (1:500; BIO-RAD, Feldkirchen, Germany, MCA1477) as a marker for T lymphocytes, as primary antibodies in 10% bovine serum albumin/PBS and 0.3 Triton. The sections were incubated overnight at 4°C with the primary antibodies. Followed by incubation, visualization was performed with Cy3-labelled donkey anti-goat Ab (1:50; Dianova 705-165-147), Alexa Fluor 488–labelled donkey anti-rabbit Ab (1:400; Dianova 711-545-

152), Alexa Fluor 488 donkey anti-goat Ab (1:200; Jackson , West Grove, PA 705-545-147), and Cy3 goat anti-rat Ab (1:100; Dianova 7112-165-167). Nuclei were stained with 1.5 μg/mL 4′,6-diamidino-2-phenylindole dilactate (DAPi), and Vectashield (Vectorlabs, Eching, Germany, H-1000-10) was used as a mounting medium. Quantification of ULBP+ subepidermal plexus (SEP) and $CD56^+$ nucleated cells was performed by an independent investigator blinded to subject group allocation on an ApoTome microscope (Zeiss, Oberkochen, Germany), equipped with an AxioCam MRm camera (Zeiss). The area of interest was 100 μm from the dermis–epidermis border. All the assessments were performed with a 40× objective and optical sectioning. Images were Z-stacked to follow the nerve fibers and NK cells. Segments of SEP located in the zone of 100 μm from the basement membrane were counted regardless of their length. Then, each segment was checked for ULBP positivity one by one as the ratio and degree of ULBP staining of SEP could show variability even within a section. Then, the percentage of ULBP-positive SEP was calculated by dividing the number of ULBP-stained SEPs by the number of total SEPs. The $CD56^+$ NK cells, which were alone or in contact with subepidermal neural plexus segments, were counted manually. As Schwann cells also expressed CD56, the cells that had a typical ellipsoid nucleus along the nerve fibers were excluded.

2.3. Data analysis

2.3.1. Flow cytometry

Flow cytometry data were compensated using FlowJo v10.7.3 (Treestar, Ashland, OR). Cells of interest were identified as per the gating strategy described in Supplementary Figure 1A (available at http://links.lww.com/PAIN/B515). Data from all 5 flow cytometry panels were analyzed using VoPo.[78] In brief, the cells were clustered into coherent subpopulations based on the expression of all cell phenotype markers using a robust bootstrapped meta-clustering algorithm. A random forest algorithm was applied to the data set composed of all cell cluster features to estimate the magnitude of differences in features separating the controls from the patients. Using a leave-group-out cross-validation procedure, the random forest model predicted the probability that each sample belonged to the patient group. The *P* value (*P*) from a Wilcoxon rank-sum test was used to test the null hypothesis that the predicted probabilities of a sample to be from a patient or control were equal. As this approach does not rely on an individual cell-to-population partition but instead integrates multiple clustering solutions to predict patient phenotype, it has less variability compared with individual solutions. Finally, cells were projected using t-distributed Uniform Manifold Approximation and Projection (t-UMAP) into 2 dimensions for visualization using the uwot R package v0.1.9.

2.3.2. Pathway analyses

The enrichment of immune cell type activation pathways in patients with FMS compared with controls was estimated through the transcriptomics level and genomics level approaches. Total mRNA from the whole blood was sequenced as described earlier. The transcriptomics data were mapped on the human genome GRCh37/hg19 using the STAR aligner,[25] and then featureCount was used to count reads mapped to each gene.[55] The analysis for differential gene expression between FMS and controls was performed using DEseq2[58] in each sex separately, then combined using the inverse variance-based approach proposed in METAL.[86] The combined DEseq2 result was analyzed by using fgsea[45] to infer pathway-level summaries. The fgsea algorithm assigned a *P* value for each pathway from evidence of concerted upregulation or downregulation of the pathway's genes based on the transcriptomics data.

At the genomics level, we first performed a genome-wide association study (GWAS) in the UK Biobank (UKB) cohort, under UK biobank application no. 20802.[2,79] Cases were defined as individuals reporting fibromyalgia for data field 20002 "noncancer illness code, self-reported" or with generalized pain all over the body (n = 8086) for data field 6159, whereas controls defined as individuals with no reported pain (n = 197,050) for data field 6159. The GWAS was computed using SAIGE, taking into account the disproportionate case-to-control ratio and cryptic relatedness.[92] Sex, age, genotyping array, and the largest 40 principal components were used as covariables. The second GWAS was performed in the HUNT study.[47] There, cases were defined as per the American College of Rheumatology's 2016 criteria (n = 2818) while controls as individuals with no pain (n = 16,901). The GWAS was computed using BOLT-LMM,[57] with birthyear, sex, genotyping batch, and the first 4 principal components as covariables. Single-nucleotide polymorphism–level summary results from both UKB and HUNT were used to derive gene-level summary results for each cohort using MAGMA.[24] MAGMA was used again to perform a meta-analysis of both UKB and HUNT studies, still at the gene level. Finally, the gene-level summary information was used by MAGMA to infer pathway-level summaries. The MAGMA algorithm assigned a *P* value for each pathway from evidence of association between the MAGMA-assigned gene-level *Z* scores and genes membership to the pathway. Gene-level summary data assigned by MAGMA took into account linkage disequilibrium between neighboring single-nucleotide polymorphisms.

Pathway analyses on both transcriptomics (using fgsea) and genetics (using MAGMA) data were performed using the Gene Ontology (GO)[31] (December 2020 version from http://baderlab.org/GeneSets). Pathways were restricted to circulating immune cell types for genes involved in immune cell activation and their regulation. As recommended,[74] pathways with more than 1000 genes or less than 10 genes were excluded from the analyses. We plotted the FDR-adjusted *P* values for pathways for transcriptomics and genetics and adjusted within each set separately.

2.3.3. Other data analyses

Results from NK activation assays were analyzed using mixed model analyses with age, sex, and BMI as fixed effects and batch and sample ID as random effects. R package lme4 v1.1.26 was used. Analysis of variance with the Tukey correction and the Pearson product-moment correlation was used for analyzing skin biopsy results. R package ggplot2 v3.3.2 was used for making the plot. All statistical analyses were performed using R v4.0.3. Finally, the entire experimental plan is illustrated in **Figure 1**.

3. Results

3.1. Characteristics of the Canadian cohort

Blood samples were collected from 90 participants, of whom 44 were diagnosed as patients with FMS. Similar to the previously reported prevalence of FMS,[73] 97.7% of the cases were women. Hence, most of the recruited controls were women (91.3%) to match

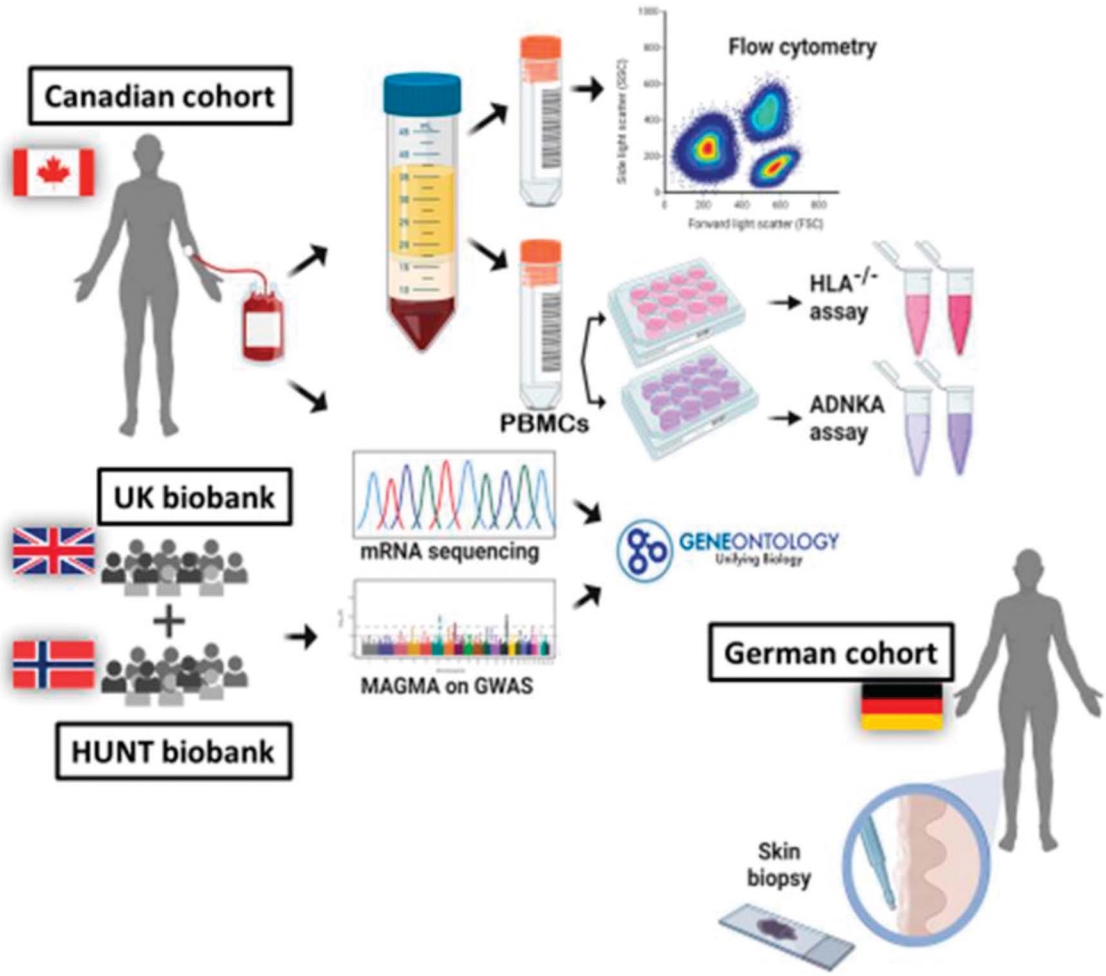

Figure 1. Experimental design. Whole blood from the Canadian cohort was split for isolating PBMCs and for mRNA sequencing. PBMCs were aliquoted for cryopreservation and used for immunophenotyping using flow cytometry and NK activation assays. mRNA was isolated and sequenced from the whole blood of the same cohort and was used for differential pathway analysis using GO database. GWAS summary results derived from the UK and the HUNT biobanks were also used for pathway analysis. Immunofluorescent staining was performed on the skin biopsies from the German cohort. ADNKA, antibody-dependent NK activation; GO, Gene Ontology; GWAS, genome-wide association study; HLA, human leukocyte antigen; NK, natural killer; PBMC, peripheral blood mononuclear cells. Created with BioRender.com.

the number of cases. Mean age and BMI did not differ significantly between the control group and the patients with FMS (**Table 1**).

Patients with FMS had significantly higher pain scores, they were in pain for a higher proportion of their days, and they had more painful body sites than the controls. Cases were 2.9 times more likely to suffer from headaches (*P* value: 0.023), 4.5 times more likely to suffer from abdominal cramps (*P* value: 0.0008), and 5.3 times more likely to suffer from depression (*P* value: 0.0005). Fatigue and poor sleep quality were more common among the cases. The detailed description of the study population is presented in **Table 1**.

3.2. Assessment of immune cell subsets in fibromyalgia

We started with analyzing differential immunophenotypes of the PBMCs in FMS compared with matched cases in a hypothesis-free manner. Flow cytometry events were selected as shown in the Supplementary Figure 1A for the downstream analyses (available at http://links.lww.com/PAIN/B515). No significant batch effects between flow cytometry runs were observed because there were no evident batch-specific cell clusters on the t-distributed stochastic neighbor embedding (t-SNE) plot (Supplementary Figure 1B, available at http://links.lww.com/PAIN/B515). Neither the total cell count nor the cell viability differed between FMS cases and controls ($t = 1.08$, P value = 0.12 and $t = 0.87$, P value = 0.66; Supplementary Figure 1, C and D, respectively, available at http://links.lww.com/PAIN/B515). Assignment of PBMC phenotypes was based on the presence of expression of various immunophenotypical markers (Supplementary Figures 2A-E, available at http://links.lww.com/PAIN/B515).

With the available choice of markers, we were able to evaluate T-helper (Th) lymphocyte subsets: Th1, Th2, Th17, and Tregs; T-cytotoxic (Tc) lymphocytes: Tc1, Tc2, and Tc17; B-lymphocyte subsets: transitional, naive, marginal zone, plasmablasts, and memory B cells; monocyte subsets: classical, intermediate, and nonclassical; DCs: conventional DC and plasmacytoid DC; NK cell subsets: $CD56^{bri}$, $CD56^{dim}$, transitional, adaptive, and terminal NK cells; and NK-like T (NKT) lymphocyte subsets: $CD4^+$ and $CD8^+$ NKTs. The 5 flow cytometry panels, namely, cyto panel, chemo panel, Treg panel, BMD panel, and NK panel had 10, 12, 11, 14, and 14 markers, respectively, apart from viability staining. To quantify novel immune cells, unsupervised gating strategies were implemented, which in theory, could explore 39,936 immune cell subsets (with 10, 12, 11, 14 and 14

Table 1

Characteristics of the Canadian cohort.

	Controls (n = 46)	Cases (n = 44)	Total (N = 90)	*P*
Age				0.960
Mean (SD)	55.6 (9.7)	55.5 (8.3)	55.6 (9.0)	
Range	40.0 - 77.0	40.0 - 73.0	40.0 - 77.0	
Gender				0.361
Women	42 (91.3%)	43(97.7%)	85 (94.4%)	
Men	4 (8.7%)	1 (2.3%)	5 (5.6%)	
BMI				0.170
N-Miss	3	2	5	
Mean (SD)	26.4 (5.8)	28.4 (7.8)	27.3 (6.6)	
Range	17.9 - 40.9	18.8 - 57.1	17.9 - 57.1	
Highest pain*				< 0.001
Mean (SD)	2.7 (6.3)	24.9 (19.9)	13.5 (18.3)	
Range	0.0 - 28.5	1.0 - 90.0	0.0 - 90.0	
Average pain*				< 0.001
Mean (SD)	2.4 (4.5)	17.6 (13.6)	9.7 (12.5)	
Range	0.0 - 24.0	0.0 - 52.5	0.0 - 52.5	
Lowest pain*				< 0.001
Mean (SD)	1.0 (2.7)	6.7 (8.8)	3.8 (7.0)	
Range	0.0 - 15.0	0.0 - 50.0	0.0 - 50.0	
% day in pain†				< 0.001
Mean (SD)	37.3 (44.6)	96.2 (17.6)	65.7 (45.2)	
Range	0.0 - 100.0	12.0 - 100.0	0.0 - 100.0	
No. of painful sites†				< 0.001
N-Miss	0	1	1	
Mean (SD)	2.8 (3.0)	13.0 (3.5)	7.7 (6.1)	
Range	0.0 - 12.0	5.0 - 19.0	0.0 - 19.0	
Headache†				0.023
No	20 (44.4%)	9 (21.4%)	29 (33.3%)	
Yes	25 (55.6%)	33 (78.6%)	58 (66.7%)	
Abdominal cramps†				< 0.001
No	29 (64.4%)	12 (28.6%)	41 (47.1%)	
Yes	16 (35.6%)	30 (71.4%)	46 (52.9%)	
Depression†				< 0.001
No	38 (84.4%)	21 (50.0%)	59 (67.8%)	
Yes	7 (15.6%)	21 (50.0%)	28 (32.2%)	
Fatigue†				< 0.001
No problem	12 (26.7%)	0 (0.0%)	12 (13.8%)	
Slight or mild problem	25 (55.6%)	5 (11.9%)	30 (34.5%)	
Moderate problem	2 (4.4%)	7 (16.7%)	9 (10.3%)	
Severe problem	6 (13.3%)	30 (71.4%)	36 (41.4%)	
Trouble thinking/remembering†				< 0.001
No problem	25 (55.6%)	1 (2.4%)	26 (29.9%)	
Slight or mild problem	17 (37.8%)	16 (38.1%)	33 (37.9%)	
Moderate problem	0 (0.0%)	2 (4.8%)	2 (2.3%)	
Severe problem	3 (6.7%)	23 (54.8%)	26 (29.9%)	
Overall sleep quality‡				< 0.001
Very good	11 (24.4%)	3 (7.1%)	14 (16.1%)	
Fairly good	26 (57.8%)	9 (21.4%)	35 (40.2%)	
Fairly bad	7 (15.6%)	17 (40.5%)	24 (27.6%)	
Very bad	1 (2.2%)	13 (31.0%)	14 (16.1%)	
Waking up tired (unrefreshed)†				< 0.001
No problem	19 (42.2%)	1 (2.4%)	20 (23.0%)	
Slight or mild problem	19 (42.2%)	2 (4.8%)	21 (24.1%)	
Moderate problem	2 (4.4%)	6 (14.3%)	8 (9.2%)	
Severe problem	5 (11.1%)	33 (78.6%)	38 (43.7%)	

* Global assessment of pain (GAP).

† Patient self-report survey for the assessment of fibromyalgia.

‡ Component 1 of PSQI.

BMI, body mass index; GAP, global assessment of pain; N-Miss, no. of missing data points; PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation.

markers per panel, $2^{10} + 2^{12} + 2^{11} + 2^{14} + 2^{14}$ marker combinations are possible). Single cells across samples were projected into 2 dimensions using t-distributed UMAP for all the panels (**Fig. 2**). Among all the PBMCs investigated, NK cell subsets were best able to differentiate FMS cases and controls (P value $< 10^{-4}$). Specifically, there were significantly fewer circulating CD56^{bri} NK cells in patients with FMS (**Fig. 2E**), although both the major circulating NK cell subsets, CD56^{bri} and CD56^{dim}, were depleted in FMS. These findings were confirmed through a traditional manual gating approach (Supplementary Figures 3A-P for major immune cell subsets and Supplementary Figure 4 for NK cell counts and percentages, available at http://links.lww.com/PAIN/B515).

3.3. State of circulating natural killer cells in fibromyalgia

As depletion of circulating NK cells was associated with FMS, we examined a number of the surface markers on NK cells: CD16, CD107a, TIGIT, CD96, CD226, CD158e, CD159a, CD159c, and CD314. We observed differences between patients with FMS and controls in the expression of CD16, CD107a, TIGIT, CD96, and CD226 with type I error < 5%. Specifically, circulating NK cells had decreased surface expression of resting NK cell markers, namely, CD16, CD96, and CD226 and increased surface expression of degranulation marker, CD107a, and exhaustion marker, TIGIT (**Fig. 3**), suggesting a hyperactive and exhausted state of circulating NK cells in FMS.

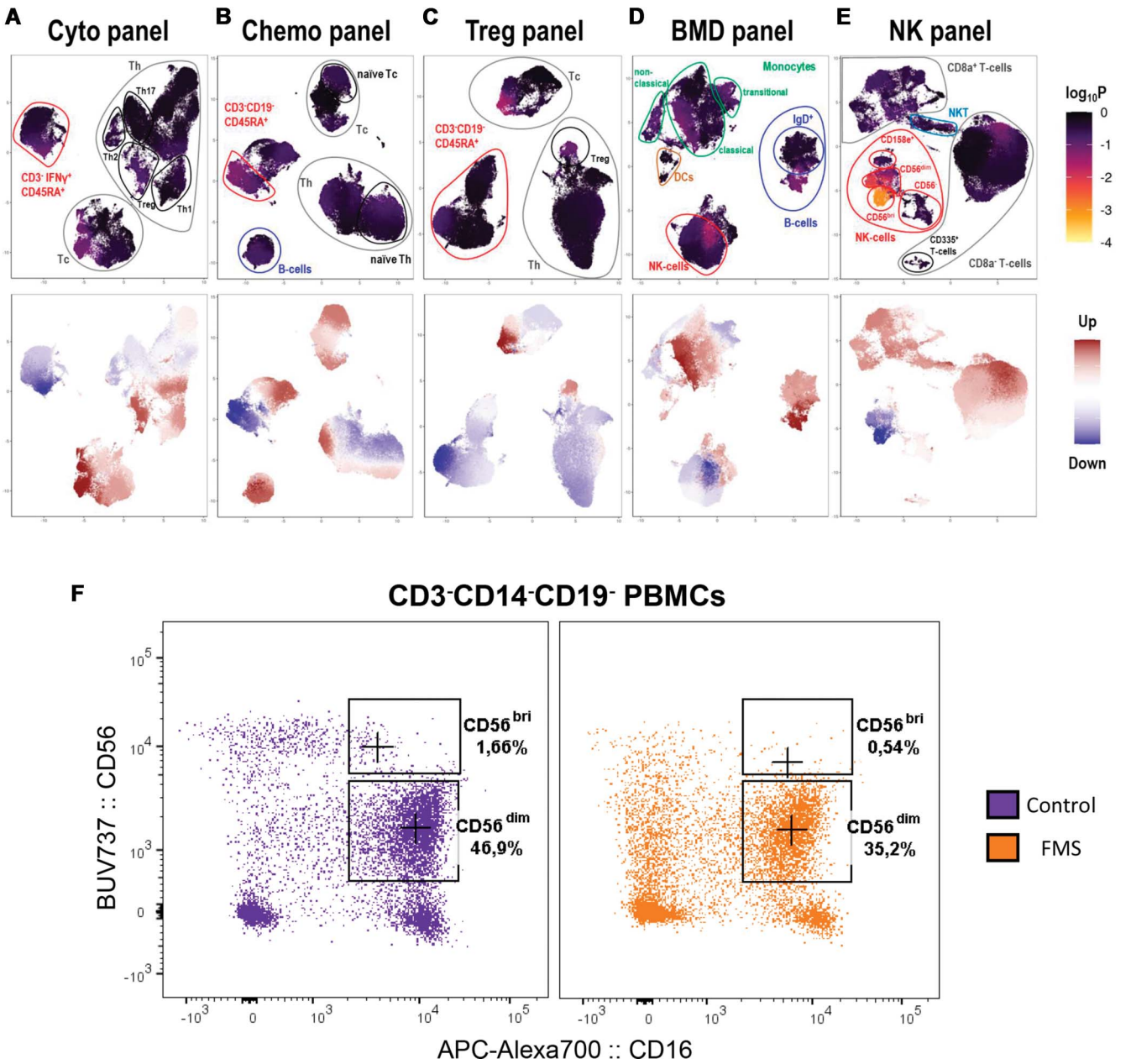

Figure 2. Differential abundance of immune cells in FMS. Single-cell data from the cyto (A), chemo (B), Treg (C), BMD (D), and NK (E) flow cytometry panel were projected onto 2 dimensions using t-distributed UMAP. Cells were colored by their computed differentiation score, which depicts the degree of association with FMS, where the lighter the color, the more significant the association. The frequency differences between cases and controls are shown in the lower figure panels where red and blue represent increased and decreased frequencies in the cases, respectively. The P values (displayed as $\log_{10}P$) were derived using the Wilcoxon rank-sum test to quantify the extent of frequency differences between control and FMS groups. (F) Representative flow cytometry plots illustrating major NK cell subsets (CD56^{bri} and CD56^{dim}) between FMS case (yellow) and control (purple). Percentages represent the proportion of alive single CD3$^-$ CD14$^-$ CD19$^-$cells. FMS, fibromyalgia syndrome; NK, natural killer.

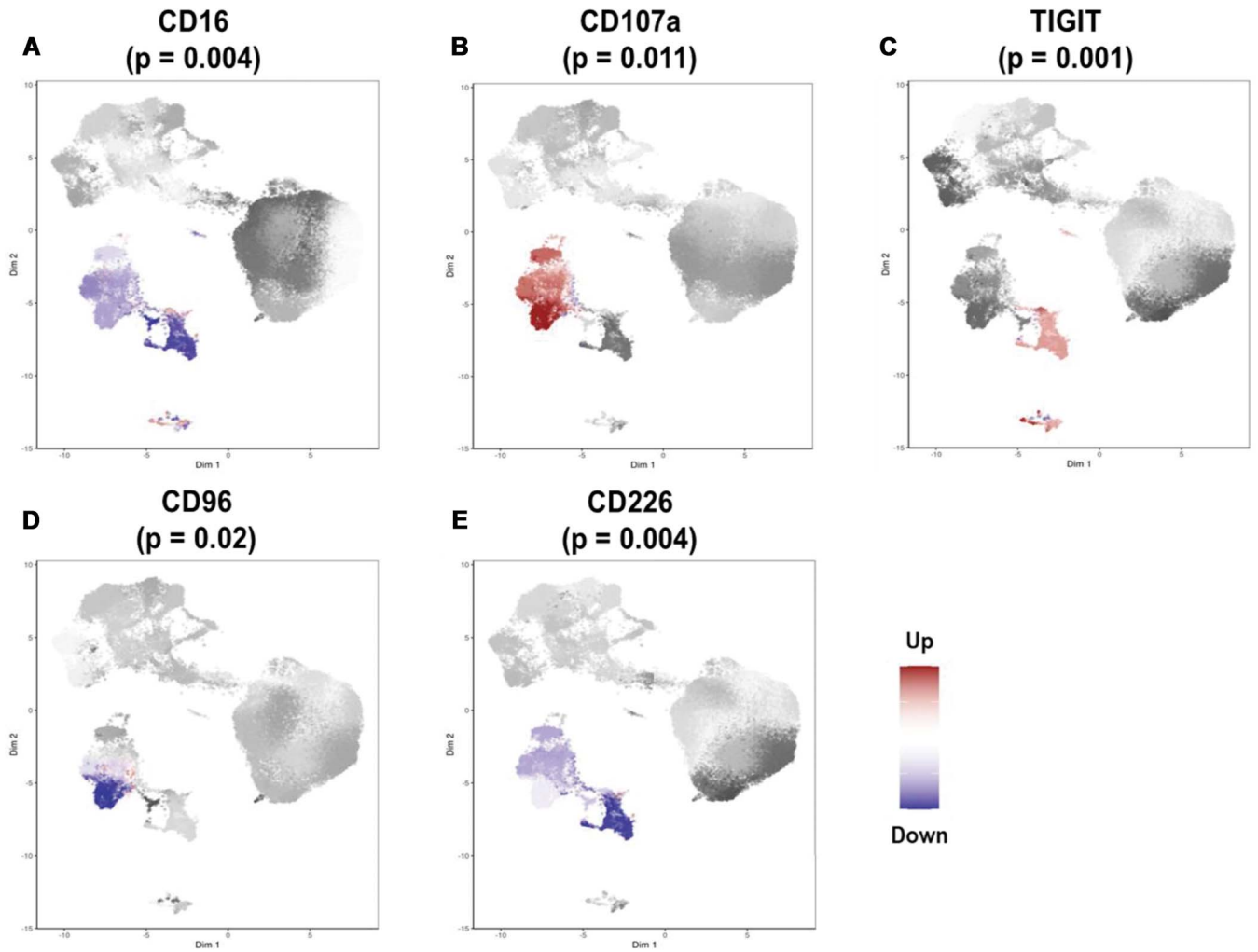

Figure 3. Differential states of NK cell subsets in FMS. The direction of differences in expression between case and control samples for CD16 (A), CD107a (B), TIGIT (C), CD96 (D), and CD226 (E). Red and blue represent increased and decreased expression in the cases, respectively. The *P* values were derived using the Wilcoxon rank-sum test to quantify the marker expression differences between control and FMS groups. FMS, fibromyalgia syndrome; NK, natural killer.

Next, we investigated whether the in vitro activation profile of the circulating NK cells from patients with FMS differed from that of the controls. Natural killer cells were assessed for their capacity to respond to 2 distinct activating stimuli. Coculturing with $HLA^{-/-}$ cells stimulates NK cells to reveal their direct functional cytotoxic potential, whereas ADNKA measures NK cell activation after incubation with antibody-bound target cells. Viable $CD3^{-}CD14^{-}CD19^{-}CD16^{+}CD56^{+}$ cells were identified as NK cells for these assays (Supplementary Figure 5A, available at http://links.lww.com/PAIN/B515). CCL4, CD107a, and interferon-gamma (IFNγ) production were used as activation outcome measures. There were no differences in CCL4+, CD107a+, or IFNγ+ NK cell proportions between FMS cases and controls in unstimulated samples (*t*-stat = 1.33, 0.98, and 0.18 and *P*value = 0.19, 0.33, and 0.86 for CCL4+, CD107a+, and IFNγ+ NK cells, respectively; Supplementary Figure 5B, available at http://links.lww.com/PAIN/B515). By contrast, NK cell activation after culture with $HLA^{-/-}$ cells revealed a significant increase in $CCL4^{+}$ (β = 13.98, *P* value = 0.02; **Fig. 4A**) and $CD107a^{+}$ (β = 17.04, *P* value = 0.0006, **Fig. 4B**) in NK cells from patients with FMS compared with controls. IFNγ+ NK cells did not differ between FMS cases and controls (β = 0.13, *P* value = 0.96, **Fig. 4C**). A typical response of stimulated and unstimulated cells for CCL4, CD107a, and IFNγ production is shown in Supplementary Figure 5C, available at http://links.lww.com/PAIN/B515. The ADNKA assay did not show statistically significant differences between cases and controls, although the trend of NK cell hyperactivity in FMS cases was still present as indicated by CCL4 and CD107a expression (β = 2.00, 10.27, and −1.63 and *P* value = 0.67, 0.10, and 0.55 for $CCL4^{+}$, $CD107a^{+}$, and $IFN\gamma^{+}$ NK cells, respectively; **Figs. 4D–F**).

3.4. Enriched immune cell activation pathways in fibromyalgia

To corroborate our findings of circulating NK cell activation in FMS, we performed pathway analysis at transcriptomics and genetic levels. mRNA was extracted from the whole blood of patients with FMS and controls of the Canadian cohort and subjected to next-generation sequencing. Pathway analysis was performed on the differentially expressed genes to test for enrichment of immune cell activation pathways. At the whole-blood transcriptomics level, subjects with FMS showed significant positive enrichment from the regulation of NK cell activation pathway (GO ID: 0032814, enrichment score = 0.5, FDR = 4%), but none of the other cell types crossed the FDR threshold of 10%. The leading edge genes of this analysis were *IL15*, *PIBF1*, *FLT3LG*, *CLNK*, *AXL*, *RHBDD3*, *TICAM1*, *TOX*, *GAS6,* and *BLOC1S3*. We then repeated pathway analysis at the genetic

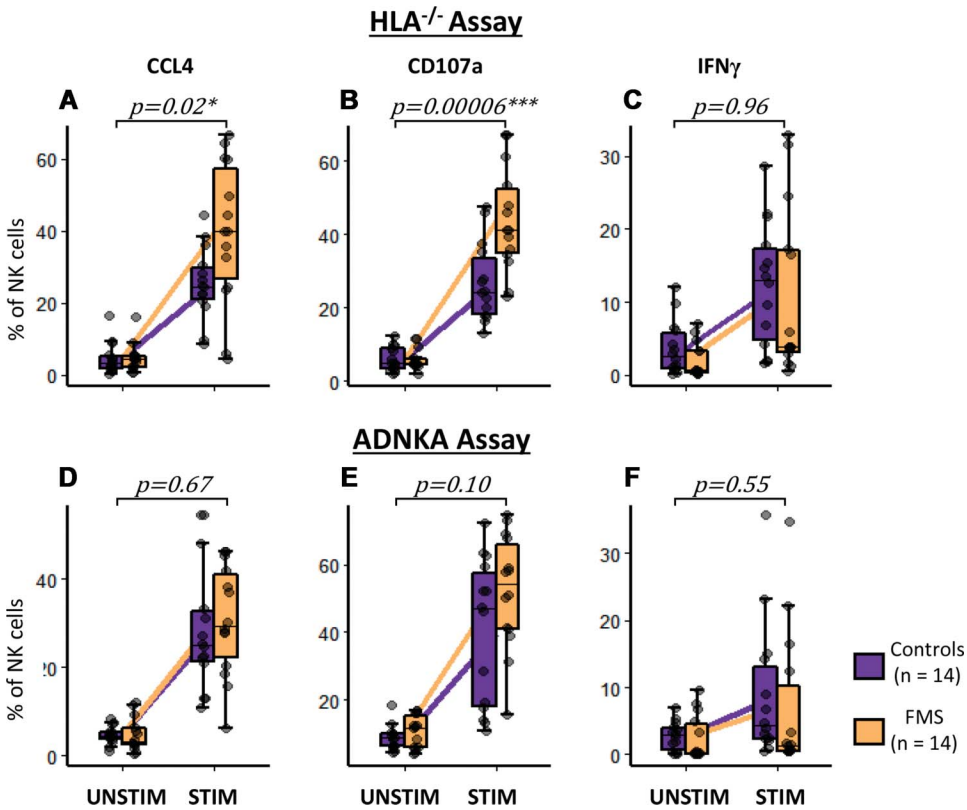

Figure 4. *In vitro* NK cell activation in FMS and control subjects. The NK cells were cocultured with either HLA$^{-/-}$ cell line (A–C) or opsonized HIV+ cells (ADNKA assay, D–F). Changes between UNSTIM and STIM NK cells in the expression of NK activation markers, CCL4 (A and D), CD107a (B and E), and IFNγ (C and F), are shown. Purple and yellow boxplots represent controls and FMS cases, respectively. Whiskers represent the interquartile range, and horizontal black lines represent group medians. Purple and yellow lines connect group means of controls and FMS cases, respectively. *P* values represent the interaction term: condition x case status of the mixed model with age, sex, and BMI as fixed effects and batch and sample ID as random effects. *$P < 0.05$ and ***$P < 0.0001$. ADNKA, antibody-dependent NK activation; BMI, body mass index; FMS, fibromyalgia syndrome; HLA, human leukocyte antigen; STIM, stimulated; UNSTIM, unstimulated.

level using summary statistics from the meta-analysis of GWAS results from the UKB and HUNT cohorts. Subjects with FMS showed significant enrichment for the NK cell activation pathway (GO ID: 0030101, FDR = 8%). The leading edge genes of this analysis were *CD2*, *IL2*, *BAG6*, *SP3*, *IFNB1*, *IFNA2*, *IFNA5*, *KLRK1*, *IL18*, and *IFNA14*. In summary, pathway analyses revealed enrichment of NK cell activation and its regulation at genomics and transcriptomics levels. The results of pathway analyses are summarized in **Figure 5**.

3.5. Peripheral recruitment of natural killer cells in fibromyalgia

We next investigated whether a decrease in circulating NK cells was a result of an overall reduction of NK cells in patients with FMS or tissue redistribution. As NK cells can get recruited to damaged peripheral nerves[22] and a considerable number of patients with FMS show reduced intraepidermal nerve fiber density,[29,36,63] we hypothesized that a decrease in circulating NK cells combined with an exhausted profile in the remaining population may be associated with their recruitment to and consequent degeneration of peripheral nerves in patients with FMS. Using skin biopsies of FMS cases and matching controls from a cohort collected at the University of Würzburg, we found increased expression of the NK activation ligand, ULBP (ligand for CD314 or NK group 2D, NKG2D, receptor), in the dermal nerve fibers of patients with FMS (**Figs. 6A and B**). Moreover, recruitment of NK cells (nucleated CD56$^+$ cells) near dermal nerve fibers was seen predominantly in the skin biopsies of patients with FMS, but not in controls (**Figs. 6C and D**). CD3 immunofluorescence was used to differentiate NK cells from T lymphocytes, and no overlap between the stains was found (data not shown). Note that ULBP is also expressed by keratinocytes[40] (**Figs. 6A and B**) and subepidermal nerves are expected to express CD56[70] (**Figs. 6C and D**), which complicates the interpretation of images.

When evaluated quantitatively, we confirmed that there were significantly more ULBP+ SEP segments in patients with FMS (**Fig. 6E**, $t = 3.4$, *P*-value = 0.003). Interestingly, this increased ULBP expression in FMS was not associated with the diagnosis of IENF deficiency (*P* value = 0.91, Supplementary Figure 6A, available at http://links.lww.com/PAIN/B515). Although recruitment of NK cells was not significantly associated with the FMS case status (**Fig. 6F**, $t = 1.6$, *P* value = 0.13), it correlated with neuronal ULBP expression (**Fig. 6G**, $\rho = 0.48$, *P* value = 0.01), Furthermore, both ULBP expression and NK cells recruitment were correlated with the FSQ scores (**Fig. 6H**, $\rho = 0.48$, *P* value = 0.01; **Fig. 6I**, $\rho = 0.36$, *P* value = 0.04, respectively).

4. Discussion

Multiple studies have reported alterations in several immune cell types in FMS in addition to nervous system contribution.[5,8,42–44,60,61,69,71] Although several mechanisms for

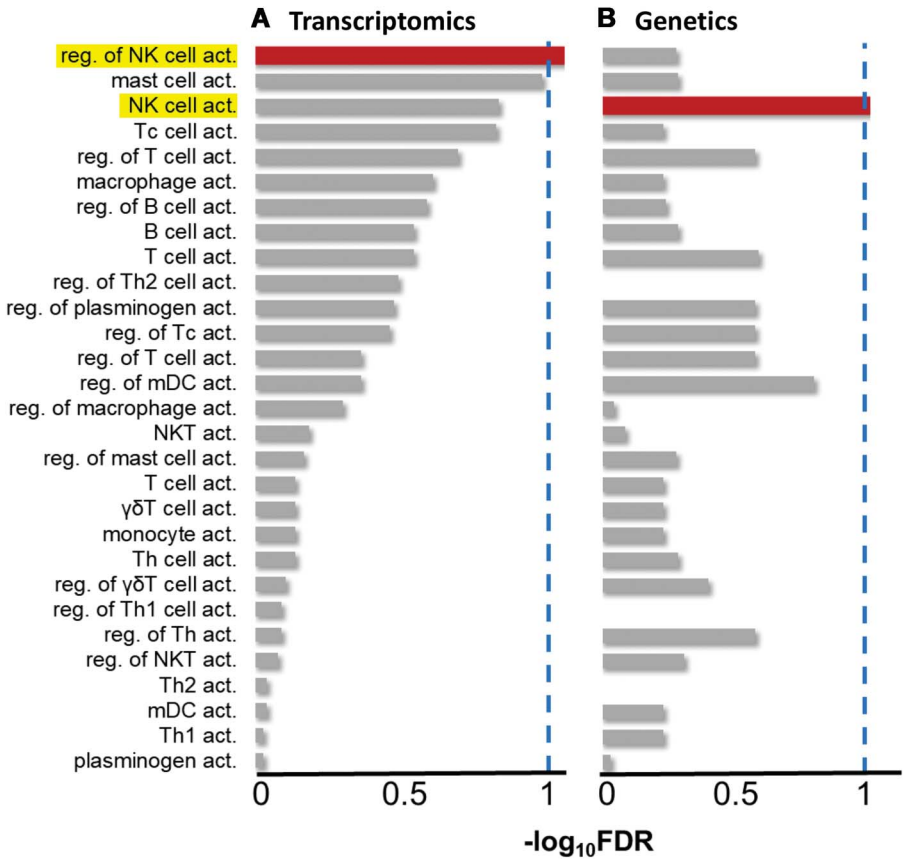

Figure 5. Enrichment of immune cell type activation pathways in patients with FMS compared with healthy controls. (A) Enrichment at the transcriptomics level, from RNA-seq data of whole blood from the Canadian cohort. (B) Enrichment at the genetic level from the meta-analyzed genome-wide association studies in the UKB and the HUNT. Dashed blue lines represent an FDR threshold of 10%. act., activation; FDR, false discovery rate; mDC, myeloid dendritic cells; NK, natural killer; NKT, NK-like T cells; reg., regulation; Tc, cytotoxic T cells; Th, helper T cells; UKB, the UK biobank; γδ, gamma-delta.

the immunopathology of FMS have been proposed, no consensus has been reached. A recent meta-analysis showed upregulated immune-inflammatory and compensatory immune-regulatory systems in FMS, but the precise involvement of the immune system in the pathophysiology of FMS is unclear.[3] Here, we used a hypothesis-free, unbiased, multiparametric flow cytometry approach on PBMCs to evaluate immune cell subsets in a case-control manner. We found that the circulating NK cells display the strongest and most robust difference between the FMS cases and controls. Although both major circulating subsets of NK cells, $CD56^{dim}$, and $CD56^{bri}$ (Supplementary Figure 4C-D, available at http://links.lww.com/PAIN/B515) were less frequent, our most significant finding was the depletion of $CD56^{bri}$ NK cells in FMS ($t = -3.88$, P value $= 0.0002$). This subset of NK cells is known for its inflammatory and immune-regulatory functions.[48,72] The $CD56^{bri}$ NK cell subset has also been shown to play a role in different disease states, such as cancer, neuroinflammation, and infection, and has been associated with tissue-specific recruitment in multiple autoimmune diseases.[33,67] When the activation state of circulating NK cells was explored, we found that they expressed a unique signature in patients with FMS characterized by decreased CD16 and higher CD107a and TIGIT expression compared with control samples. Similar to CD16 expression, CD226 (DNAX accessory molecule-1, DNAM-1) and CD96 (T cell-activated increased late expression protein, TACTILE) were decreased on the surface of the circulating NK cells. This phenotype is typically associated with a state of chronic activation and exhaustion in circulating NK cells.[41,62,75] In addition, our in vitro studies indicated that NK cells from patients with FMS were hyperresponsive. They produced more CCL4 (also known as macrophage inflammatory protein-1β, MIP-1β) and showed increased degranulation (as evident by increased CD107a expression) as compared with the NK cells from the controls when cocultured with $HLA^{-/-}$ cells. By contrast, NK cells from patients with FMS responded poorly to antibody-dependent signaling in an ADNKA assay. This may be related to the downmodulation of CD16 on NK cells from FMS cases because CD16 is the receptor for the Fc portion of antibodies and crucial in mediating antibody-dependent NK cell activation. Concordantly, pathway analyses of our RNA-seq transcriptomics data and GWAS-based genetic meta-analysis in 2 other independent cohorts confirmed enrichment for NK cell activation and its regulation profiles in patients with FMS (**Fig. 5**).

Because of the paucity of circulating NK cells, we questioned where the circulating NK cells could be redistributed and hypothesized that they could be recruited towards the peripheral nerves in patients with FMS. This hypothesis was based on the high prevalence of small fiber neuropathy in patients with FMS which ranges from 45% to 59%, depending on the methods used for determination[36,63] and a variable degree of intraepidermal nerve fiber loss.[83] In humans, the NK activation receptor CD314 (alias, NKG2D) recognizes 2 families of major histocompatibility complex (MHC) I-like molecules, MHC I-related chain (MIC) A and B, and the ULBPs (1-6).[9] The expression of these ligands is restricted in normal tissues; however, infected, malignant, or stressed cells overexpress ULBPs, MICA, and MICB.[35] Although until recently virtually no studies were

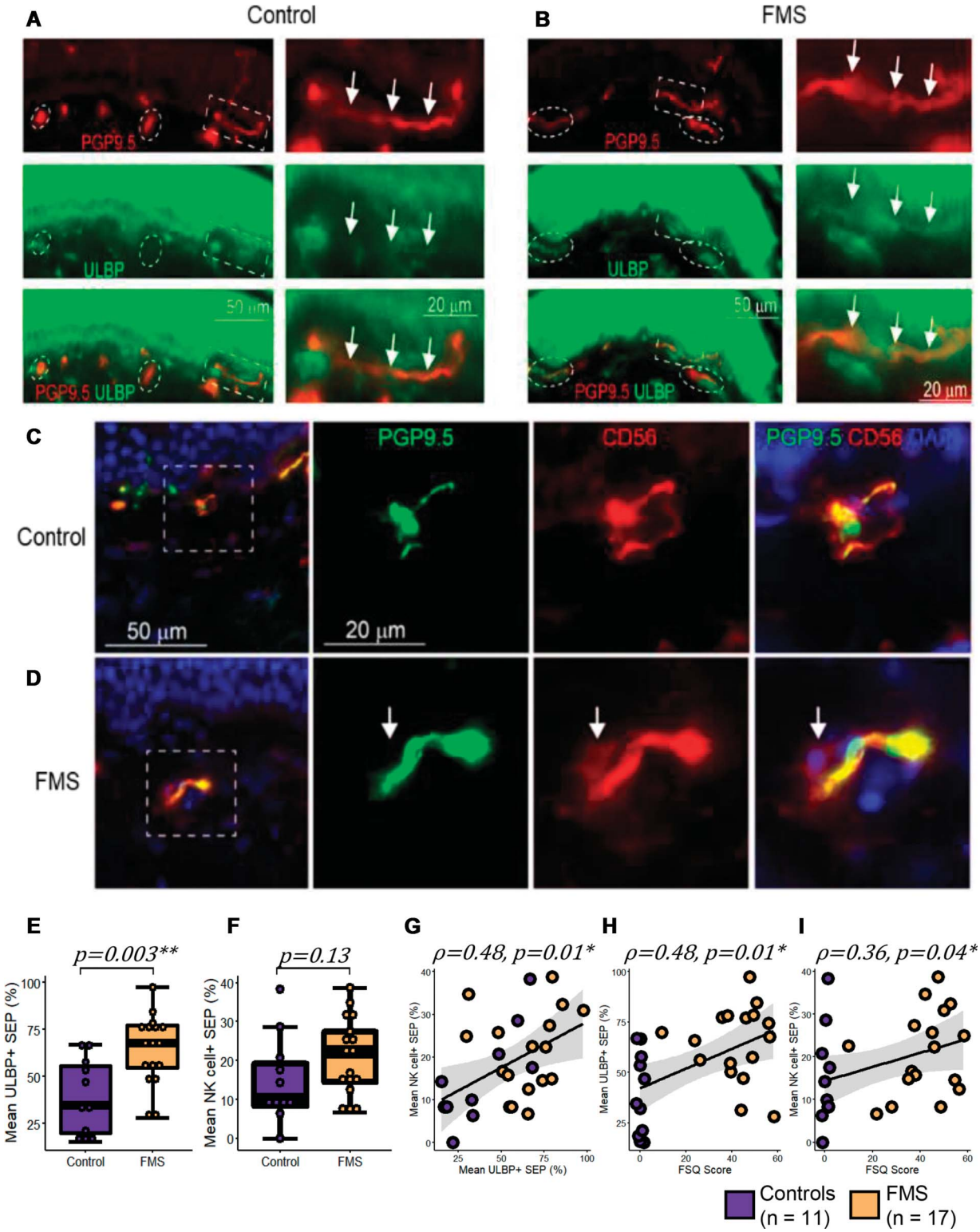

Figure 6. ULBP expression and NK cell recruitment at the dermal nerve fibers in FMS. (A and B) Representative immunostaining of the skin from (A) a control and (B) an patient with FMS with nerve fibers stained with anti-PGP9.5 (red) and NK activation ligand stained with anti-ULBP (green). A high magnification image of the area in the white dashed box shows costaining (marked with arrows) of PGP9.5 and ULBP in FMS, but not in control. White dashed ovals show SEP. (C and D) Microimages from a control (C) and an patient with FMS (D) stained for PGP9.5 (green), nuclei (DAPI in blue), and CD56 (red). Arrows mark NK cells seen in the proximity of a SEP in FMS, but not in controls. Boxplots showing the distribution of (E) ULBP+ SEP and (F) SEP with NK cells, stratified by case status. Whiskers represent the interquartile range and horizontal black lines represent group medians. (G–I) Correlation between (G) ULBP expression on SEP and NK cell recruitment at SEP, (H) ULBP expression on SEP and FSQ scores, and (I) NK cell recruitment at SEP and FSQ scores. Purple and yellow depict controls (n = 11) and FMS cases (n = 17), respectively. Linear regression and its 95% confidence interval are shown as a black line and gray shaded area, respectively. *P* values were calculated using the Welch 2-sample *t* test and Spearman rank correlation. $^{*}P < 0.05$ and $^{**}P < 0.01$. FMS, fibromyalgia syndrome; FSQ, Fibromyalgia Survey Questionnaire; NK, natural killer; PGP, protein gene product; SEP, subepidermal plexus; ULBP, UL16-binding protein.

exploring the interaction of NK cells and peripheral nerves, extravasation of circulating NK cells and its recruitment have been recently shown to be associated with partially damaged peripheral nerves in mice.[22] In support of our hypothesis, we identified a higher expression of ULBP on the dermal nerve fibers of patients with FMS from an independent cohort (**Fig. 6E**). Furthermore, both higher expression of ULBP on and recruitment of NK cells to the dermal nerve fibers were significantly associated with FSQ score (**Figs. 6H, I**). In addition, we found that the recruitment of NK cells to the dermal nerve fibers of these patients correlated with ULBP expression (**Fig. 6G**). Being derived from an independent FMS cohort, these results support our initial discovery and suggest a systemic redistribution of NK cells from the circulation to the dermal nerve fibers in patients with FMS.

In line with these observations, decreased circulating NK cells have been reported previously in FMS[17,49,50] and in a related chronic fatigue syndrome with tender points.[68] NK cells from patients with FMS express more adhesion molecules, CD11b, and CD49d, compared with controls, which would promote their recruitment or retention at peripheral sites.[60] Furthermore, NK cell activity has been shown to correlate negatively with right hemisphere activity in the secondary somatosensory and motor cortices and thalamus and bilaterally related to activity in the posterior cingulate cortex in FMS.[53] Decreased numbers of circulating NK cells also has been reported in other chronic pain conditions such as chronic lower back pain,[10,21,84] cluster headache,[13] and vulvar vestibulitis.[65] Thus, redistribution of NK cells may be a general mechanism in chronic pain conditions.

Combining our results with previous studies, we propose a new heuristic model of the immunopathology of FMS (**Fig. 7**). According to our model, in FMS, peripheral nerves chronically express NK activation ligand(s), leading to the extravasation and peripheral recruitment of circulating NK cells. This extravasation leads to a reduced number of circulating NK cells in the blood. Notably, ULBP expression on the intraepidermal nerve fibers is associated with FMS irrespective of IENF deficiency (Supplementary Figure 6A, available at http://links.lww.com/PAIN/B515) and there is only a weak anticorrelation between ULBP expression and IENF density ($\rho = -0.35$, P value $= 0.07$, Supplementary Figure 6G, available at http://links.lww.com/PAIN/B515). However, because of the significant association of ULBP expression with FMS severity ($\rho = 0.48$, P value $= 0.01$, **Fig. 6H**), it is possible that in some patients with FMS even when the nerves are being marked by ULBP for removal by NK cells, they continue to regrow, masking IENF deficiency but not preventing pain symptoms. Alternatively, nerve damage and regrowth could be taking place periodically, thus explaining the waxing and waning nature of FMS symptoms.[1] Nonetheless, the site of this neuro-immune interaction between NK cells and dermal nerve fibers could justify some of the dermatological manifestations of FMS,[51] such as lichen simplex chronicus, neurotic excoriations, prurigo nodules, pruritus, burning sensations, and hyperhidrosis. It is unclear if ULBP expression on the peripheral nerves is a cause, consequence, or exacerbator of the nerve damage in FMS. In addition, we do not know if ULBP is the only NK activation ligand expressed by the peripheral nerves in FMS. Alternatively, FMS could be a manifestation of increased genetic predisposition of NK cells for overactivation (**Fig. 5B**) which then leads to peripheral nerve damage and enhanced ULBP expression, triggering a vicious cycle.

Usually, the expression of NK activation ligands is a signal of cell stress and damage, such as response to viral infections[93] or injury,[23] and their expression triggers selective destruction of the marked cells. The cause of NK activation ligand (ULBP) expression on the dermal nerve fibers of patients with FMS is unknown and will require further investigation. However, at least 2 mechanisms can be proposed. First, there is evidence that viral infections can trigger FMS and chronic fatigue syndrome.[12] Thus, a chronic or latent viral infection of sensory neurons may lead to the expression of ULBP on the peripheral nerves of patients with FMS. The other possibility is related to the recently identified autoimmune mechanisms contributing to FMS through immunoglobulin G (IgG) autoantibodies that sensitize peripheral sensory neurons in patients with FMS.[34] This sensitization can also lead to NK activation ligand expression on the sensory neurons. There are substantial similarities between FMS and other autoimmune diseases,[66] and NK cells play multiple crucial roles in linking innate and adaptive immune responses to either promote or

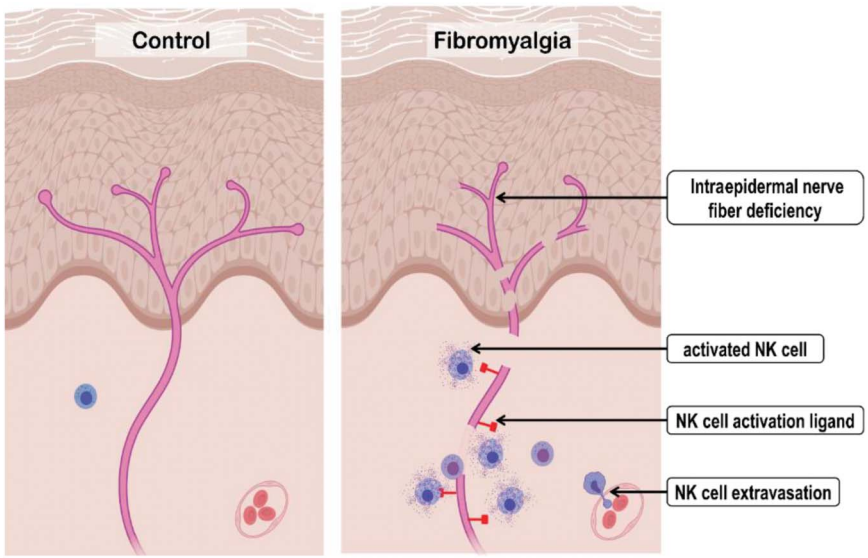

Figure 7. Heuristic model of NK cells' contribution to FMS pathogenesis. Compared with controls, patients with FMS express the NK activation ligand(s) on the peripheral nerves. This promotes extravasation, recruitment, and activation of the circulated NK cells with subsequent chronic degeneration of the peripheral nerve. Created with BioRender.com. FMS, fibromyalgia syndrome; NK, natural killer.

protect against the onset of autoimmune conditions. Decreased numbers of circulating NK cells are reported in autoimmune patients, and this alteration has been linked to their trafficking to damaged tissues.[33] Both activation and exhaustion of circulating NK cells have been reported in various chronic inflammatory conditions, such as chronic infections, malignancies, and autoimmune disorders.[85] Hyperactivation of NK cells has been also reported in multiple autoimmune diseases.[33] Nevertheless, the most important piece of this puzzle, the initial stimulus for immune cell activation in FMS, remains to be discovered.

It is important to stress that although our findings show an association between NK cells and FMS, it does not exclude the possibility that other immune cell types could also contribute to this condition in a consequent, interdependent, or independent manner. Our flow cytometry, genetics, and transcriptomics data support immune dysregulation in FMS, where NK cells' neuroimmune interaction could be a partial explanation for FMS immunopathology. Importantly, the contribution of multiple immune cells to chronic inflammation that drives neurodegeneration has been reported,[89] whereas the potential role of NK cells in neurodegeneration and pain modulation has only recently been recognized in an animal model.[22] Hence, exploring the immune system using holistic approaches such as immune cell profiling with mass cytometry and single-cell RNA sequencing in FMS over the course of disease development is needed to further explore the immune system's involvement in FMS. Similarly, the causes of NK activation ligand expression by the dermal nerve fibers in FMS warrant further examination.

To conclude, this study identified a neuroimmune interface between peripheral nerves and NK cells in patients with FMS that suggests a novel mechanism for FMS pathogenesis and a new direction in the development of therapeutic options to treat this poorly managed syndrome.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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