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Modular Transcriptional Repertoire Analyses of Adults With Systemic Lupus Erythematosus Reveal Distinct Type I and Type II Interferon Signatures

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Objective. **The role of interferon-α (IFNα) in the pathogenesis of systemic lupus erythematosus (SLE) is strongly supported by gene expression studies. The aim of this study was to improve characterization of the blood IFN signature in adult SLE patients.**

Methods. **Consecutive patients were enrolled and followed up prospectively. Microarray data were generated using Illumina BeadChips. A modular transcriptional repertoire was used as a framework for the analysis.**

Results. **Our repertoire of 260 modules, which consisted of coclustered gene sets, included 3 IFN-annotated modules (M1.2, M3.4, and M5.12) that were strongly up-regulated in SLE patients. A modular IFN signature was observed in 54 of 62 patients (87%) or 131 of all 157 samples (83%). The IFN signature was more complex than expected, with each module displaying a distinct activation threshold (M1.2 < M3.4 < M5.12), thus providing a modular score by which to stratify SLE patients based on the presence of 0, 1, 2, or 3 active IFN modules. A similar gradient in modular IFN signature was observed within patients with clinically quiescent disease, for whom moderate/strong modular scores (2 or 3 active IFN modules) were associated with higher anti–double-stranded DNA titers and lower lymphocyte counts than those in patients with absent/mild modular scores (0 or 1 active IFN modules). Longitudinal analyses revealed both stable (M1.2) and variable (M3.4 and M5.12) components of modular IFN signature over time in single patients. Interestingly, mining of other data sets suggested that M3.4 and M5.12 could also be driven by IFNβ and IFNγ.**

Conclusion. **Modular repertoire analysis reveals complex IFN signatures in SLE, which are not restricted to the previous IFNα signature, but which also involve IFNβ and IFNγ.**

Interferons (IFNs) regulate the function of most immune cells. Type I (including IFNα and IFNβ) and type II (IFNγ) IFNs are the most studied members of the family. Many cell types, especially macrophages and dendritic cells, secrete type I IFN, while type II IFN is mostly produced by T lymphocytes and natural killer cells. IFN-regulated genes (1) play a major role in controlling viral infections and nonviral inflammatory

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and autoimmune disorders, such as systemic lupus erythematosus (SLE) (2).

SLE is a chronic autoimmune disease characterized by the breakdown of tolerance to nuclear antigens, especially nucleic acids, resulting in widespread organ damage. Its broad-spectrum manifestations and waxing and waning course make evaluation of disease activity challenging for clinicians. Some laboratory parameters, including anti–double-stranded DNA (anti-dsDNA) antibody titers and complement factor levels, are often monitored, but several longitudinal studies have demonstrated their shortcomings (3), and no surrogate biomarker for disease activity has been validated to date. Hence, there is an important need to develop objective, simple, and robust SLE biomarkers (4).

Defining the molecular pathways responsible for the pathogenesis of SLE could aid diagnosis, biomarker development, and therapy. A pivotal role for type I IFN (IFNα) in the pathogenesis of SLE is strongly supported by many data, including gene expression studies (5). Over the last decade, several groups have reported increased expression of type I IFN–regulated genes in SLE (i.e., the so-called "type I IFN signature") (5,6). This discovery prompted the initiation of therapeutic trials aimed at evaluating the benefits of anti-IFNα therapy in SLE patients (7) as well as the benefits of IFN-related biomarkers or "scores" to assess SLE disease activity or response to therapy (5,6).

Although type I IFN activation has been previously correlated with SLE activity (5,6), this association has not been validated in recent longitudinal studies (8,9). This is probably because few longitudinal gene expression studies have been conducted (10), and the contribution of type II IFN to the "IFN signature" in blood and tissues may have been overlooked, as recently shown in dermatomyositis (11), Sjögren's syndrome (12), and even in SLE (13–15). In addition, because the transition from genome-wide RNA expression analysis (thousands of genes) to only a few target genes is challenging, scores may have been biased by a knowledge-driven data-reduction process.

Our group has developed an original approach based on modules that correspond to coclustered gene sets built via an unbiased data-driven approach (16). This approach has shown promising results in pediatric SLE (17). The aim of this study was to use modular transcriptional repertoire analysis to improve characterization of the blood IFN signature in adult patients with SLE.

PATIENTS AND METHODS

Ethics statements. The LUPUCE study (ClinicalTrials.gov identifier: NCT00920114) was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved in France by the Comité de Protection des Personnes Sud Méditerranée 1 (IDRCB 2009-A00257-50) and in the US by the Institutional Review Boards (IRBs) of the Baylor Institute of Immunology Research (IRB 011-173) and the Benaroya Research Institute (IRB 12085). Written informed consent was obtained from all patients enrolled prior to any study-related procedure.

Study design and classification of patients. Sixty-two consecutive patients who fulfilled the American College of Rheumatology revised criteria for SLE (18) as updated in 1997 (19) were enrolled between 2009 and 2011 in the Departments of Internal Medicine and Nephrology at a French referral center for autoimmune diseases (Hôpital de la Conception, Marseille, France) and followed up prospectively. Peripheral blood was collected by venipuncture using Tempus tubes at inclusion and at each followup visit. At each visit, disease activity was assessed using the score on the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) version of the SLE Disease Activity Index (SLEDAI) (20). Flares were defined as a SELENA–SLEDAI score of $\geq$3 points (improvement was defined as a decrease of $\leq$2 points) (21).

Immunologic analyses (determination of autoantibodies and complement fractions) were performed in the same laboratory (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Healthy volunteer donors comprised 20 adults matched for age, sex, and ethnicity (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf) who had no personal or family history of lupus or other autoimmune conditions and who were sampled once.

SLE patients were classified into 3 groups (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). The "at inclusion" group included all SLE patients at their first visit, irrespective of SLE disease activity at that time. The "quiescent disease" group included SLE patients at their first available visit who had low levels of disease activity (defined as no flare or treatment modifications for at least 60 days prior to the visit) and a SELENA–SLEDAI score of $\leq$4. The "longitudinal" group included SLE patients who had at least 3 consecutive visits during the study.

RNA preparation and microarray hybridization. RNA was processed and microarray data were generated using Illumina BeadChips as described elsewhere (22) (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Data are deposited in NCBI GEO (accession no. GSE49454; online at http://www.ncbi.nlm.nih.gov/geo/). Polymerase chain reaction (PCR) analyses were performed on the same samples using a Fluidigm Real-Time PCR platform (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf).

Microarray analyses. Analyses were performed using an R software environment (http://www.r-project.org). Background subtracted data were preprocessed with quantile nor-

malization, flooring to 10, $\log_2$ calculation, and selection of transcripts that were "present" in at least 10% of samples. Probes that passed the filters (Benjamini-Hochberg multiple testing correction with a false discovery rate [FDR] of ≤0.05 and a fold change of ≥2 or ≤1/2) were considered for further gene ontology and pathway analyses.

Modular transcriptional repertoire analyses. Analyses were performed using the second generation of a modular framework as previously described (16,17). Three of 260 modules (M1.2, M3.4, and M5.12) are annotated "Interferon." Module transcript content and annotations are available online (http://www.biir.net/public_wikis/module_annotation/V2_Trial_8_Modules).

Group-level analyses showing disease fingerprints (versus healthy baseline), as linear models, were run at the probe level using the R package Limma (23) to determine which probes were statistically significant (Benjamini-Hochberg FDR of <0.05). Positive percentages for up-regulation and negative percentages for down-regulation were calculated based on the number of statistically significant probes assigned to each module. Because probes assigned to a module typically show a consistent pattern, the percentage difference (% positive − % negative) was calculated to represent the module with one metric. The percentage difference of each of the modules was mapped on a grid, where each position corresponded to 1 of the 62 main modules. Modules containing transcripts with increased expression were represented on a red scale, while those with decreased expression were represented on a blue scale.

For individual analyses, each sample was compared to the average of the control samples for each probe. Filtering comprised a fold change (≥2) and a difference in gene expression level (≥100). The level of regulation of each module was calculated as the percentage difference: % up-regulated probes − % down-regulated probes. A module was considered "active" when the percentage difference was ≥20. A modular IFN signature was considered present if at least 1 of the 3 IFN-annotated modules was active. A modular IFN score was defined according to the number of IFN-annotated modules as absent, mild, moderate, or strong if 0, 1, 2, or 3, respectively, of these IFN-related modules were active.

Other gene expression data sets and scores. Publicly available blood gene expression profiles from independent pediatric and adult SLE cohorts (22,24) were used to validate modular IFN signatures observed in our cohort. Blood gene expression profiles from patients receiving IFNα or IFNβ (25,26) were used to evaluate the influence of various types of IFN on the modular IFN signature. Interferome version 2.0 (27), an online resource containing data on IFN-inducible genes from more than 20 in vitro studies conducted on various human cells (http://interferome. its.monash.edu.au/interferome/home.jspx), was used to identify IFN-related genes and to evaluate the weight of type I IFN versus type II IFN, as well as IFNα versus IFNβ, on the expression of these genes (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_ data.pdf). An "IFN molecular distance to health" was defined as the number of genes with a >2 fold change for each sample compared to the healthy control samples, where only genes with evidence of IFN regulation from the Interferome database were included. Previously published IFN scores, using different sets of IFN-regulated genes, were calculated from gene expression microarray data for our samples according to published algorithms (8,28–30).

Statistical analysis. Numerical data were processed and analyzed using R statistical software. For continuous data, comparisons between groups were conducted using analysis of variance (assuming normality was appropriate) or the nonparametric Kruskal-Wallis test. Student's *t*-test or Wilcoxon's test was conducted if further testing was needed to determine which group was different. Linear models were used to test for trend. For categorical variables, Fisher's exact test was used to determine differences in contingency tables, and the chi-square test for trends in proportions was used if the categorical variable was ordinal. Correlations were assessed by Pearson's (assuming normality was appropriate) or Spearman's correlation test. Random forest analysis (randomForest package from CRAN [the Comprehensive R Archive Network]) was used to build a classifier based on a 9-gene IFN panel. Other packages from CRAN were used for the bee-swarm plots (beeswarm package) and the heatmaps (lattice, latticeExtra, grid packages). A Circos plot was used to represent the longitudinal IFN modular signature (31).

RESULTS

Characteristics of the SLE patients. Details on the characteristics of the 62 SLE patients are available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf. The median age of the patients was 38 years (range 18–70 years), 85% were women, and 89% were white. The mean duration of SLE was 7.8 years (range 0–40 years). At inclusion, 97% and 63% of patients were positive for antinuclear antibodies and anti-dsDNA antibodies, respectively. Fifty-two patients (84%) were receiving oral corticosteroids, 35 patients (56%) were receiving antimalarials, and 31 patients (50%) received immunosuppressive drugs. At inclusion, 27 patients (44%) had clinically quiescent disease, while 35 patients (56%) presented with a flare, with a mean SELENA–SLEDAI score of 12 (range 4–26). The median followup time per patient was 5.9 months (range 0–28 months); data on 157 visits were collected (mean of 2.5 visits per patient [range 1–6]).

Modular repertoire analysis recapitulates whole transcriptome gene expression analyses and confirms the prevalent IFN signature in adult SLE patients. Modular repertoire analysis was performed at the group level (all SLE patient samples versus all healthy control samples) and at the individual level (each individual SLE patient sample versus all healthy control samples). At the group level, this approach identified the 3 previously described IFN modules among the most up-regulated in SLE patient samples (n = 157) compared to matched healthy control samples. There were no down-regulated probes, and 36 of 36 (100%), 56 of 62 (90%), and 49 of

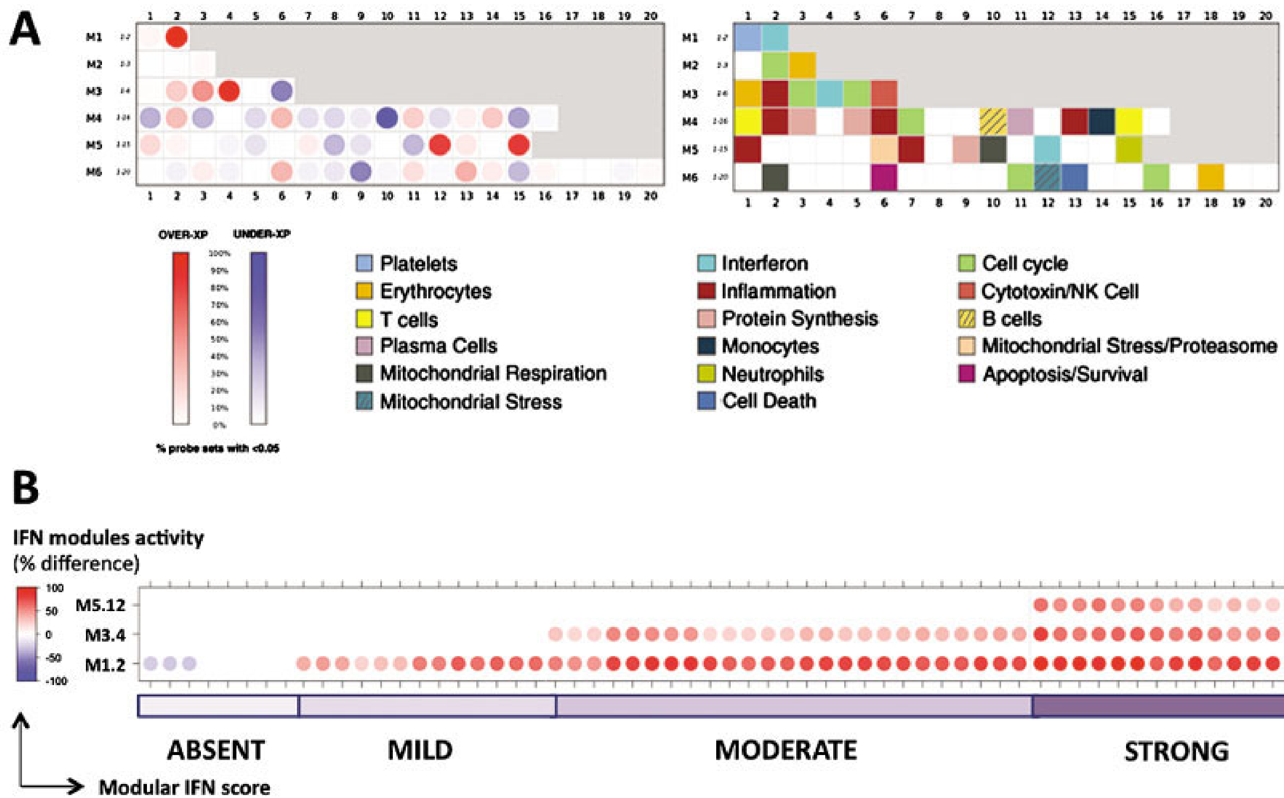

Figure 1. Modular repertoire analysis of systemic lupus erythematosus (SLE) patients compared to healthy controls. **A,** Modular analysis at the group level. Samples from SLE patients (n = 157) are compared to samples from matched healthy controls (n = 20). Each module is assigned a position on the grid. The percentage difference between probes significantly up-regulated and down-regulated within each module determines the color and intensity of the spot (red for up-regulation in SLE, blue for down-regulation). Module annotations are provided in the second grid. A strong up-regulation in SLE was observed for 4 modules, including the 3 interferon (IFN)–annotated modules M1.2 (100% of probes up-regulated in SLE patients), M3.4 (90% up-regulated, 0% down-regulated), and M5.12 (78% up-regulated, 0% down-regulated) as well as the neutrophils-annotated module (M5.15). **B,** Modular IFN signature at the individual level. Each sample from SLE patients at inclusion (n = 62) is compared to the average of samples from healthy controls (n = 20). The percentage difference between probes up- and down-regulated (fold change ≥2 or ≤1/2 and difference in gene expression [XP] level ≥100) determines the color and intensity of each IFN module for each sample. Modular IFN signature was present (at least 1 active IFN module, i.e., percentage difference ≥20) in 54 of 62 SLE patients. A modular IFN score was assigned to samples (“absent,” “mild,” “moderate,” or “strong”) according to the number of active IFN modules (0, 1, 2, or 3, respectively). NK = natural killer.

63 (78%) probes were significantly up-regulated for M1.2, M3.4, and M5.12, respectively (Figure 1A). Other modules corresponding to signatures previously established in SLE patients (e.g., neutrophil signature and its corresponding module M5.15) were also identified (Figure 1A). At the individual level, a modular IFN signature (at least 1 active IFN module) was observed in 54 of 62 SLE patients at inclusion (87%) (Figure 1B) and in 131 of all 157 samples from SLE patients (83%) (data not shown).

Results obtained using this modular approach matched those observed with a gene-level approach (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Applying published IFN scores to our cohort revealed the presence of an IFN signature (e.g., a “positive” IFN score) in 71–88.5% of the 157 SLE patient samples (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf).

Individual modular repertoire analysis reveals a dynamic modular IFN signature within and across patients. Strikingly, we observed a coordinated gradient of IFN modules across samples, each module displaying a distinct activation threshold. Indeed, when only 1 of the 3 IFN modules was up-regulated, it always corresponded to M1.2. Module M3.4 appeared next, and there was no M5.12 module up-regulation in the absence of the 2 others (Figure 1B). Samples could then be

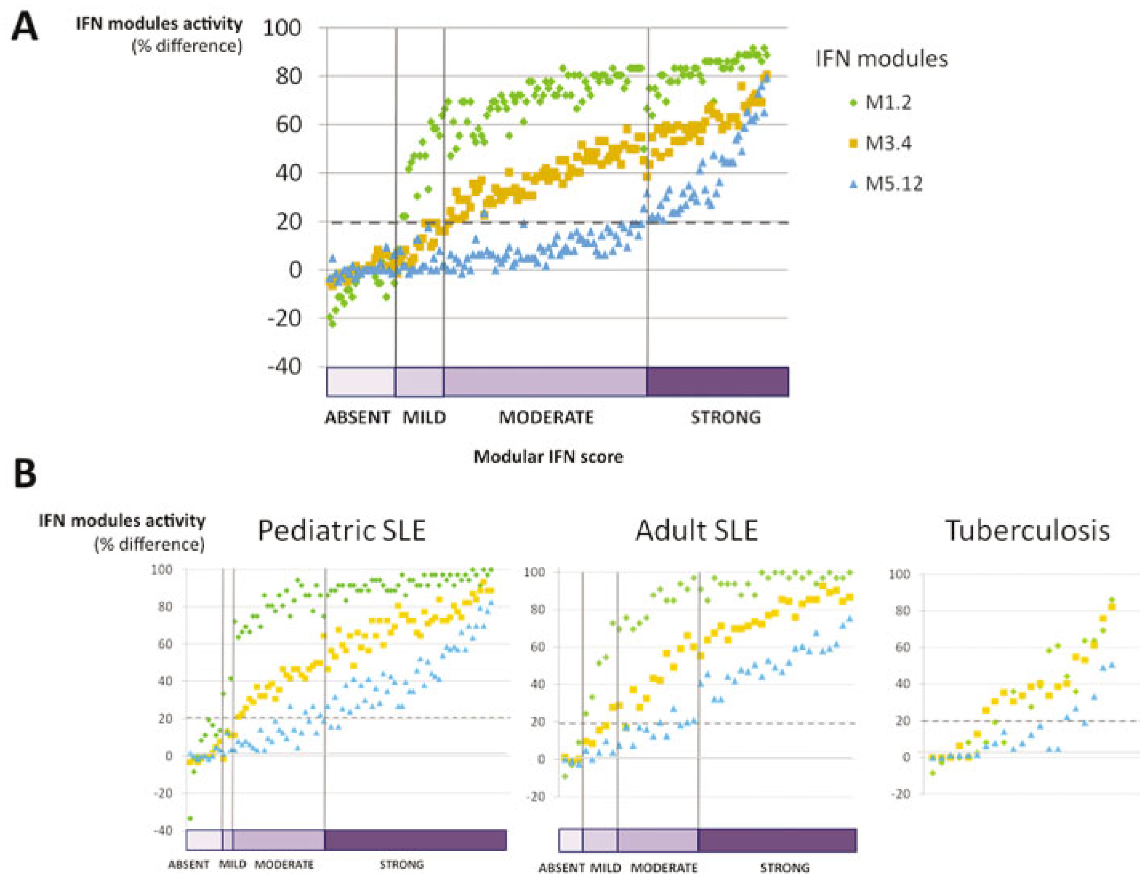

Figure 2. Repartition of samples from SLE patients according to the modular IFN score. **A,** The 157 samples from SLE patients were ordered and classified according to the modular IFN score (number of active IFN modules, from 0 to 3). The IFN signature was "absent" in 26 samples, "mild" in 17 samples, "moderate" in 68 samples, and "strong" in 46 samples. A dynamic IFN signature, from M1.2 to M3.4 and M5.12, was observed: when only 1 of the 3 IFN modules was up-regulated, it always corresponded to M1.2. Module M3.4 appeared next, and there were no M5.12 modules up-regulated in the absence of the 2 others. **B,** The same dynamic IFN signature was observed in 2 independent cohorts of SLE patients: a cohort of 82 pediatric patients (22), with Hispanic (57%), black (23%), white (15%), and Asian (5%) ethnicities, and a cohort of 43 adult patients (24), with black (54%), white (44%), and Asian (2%) ethnicities. In contrast, although a modular IFN signature was observed in patients with active tuberculosis (22), no such gradient was observed in the IFN modules. Horizontal dashed lines correspond to the 20% difference in regulation (% up-regulated probes − % down-regulated probes) above which a module was considered "active." Symbols represent individual samples. See Figure 1 for definitions.

classified according to a "qualitative" modular IFN score based on the number of active IFN modules: "absent" for no active IFN modules, "mild" for 1 active IFN module, "moderate" for 2, and "strong" for 3. Among the 62 SLE patients, only 8 (13%) had an "absent" modular IFN signature at inclusion, whereas 8 (13%), 24 (39%), and 22 (35%) exhibited "mild," "moderate," and "strong" modular IFN scores, respectively (Figure 1B). Similar patterns were observed across all samples from SLE patients (n = 157) (Figure 2A). In addition, a similar coordinated gradient of the 3 IFN modules was observed in 2 independent cohorts of SLE patients involving both children and adults, while a different gradient was observed in a cohort of patients with tuberculosis, although they did exhibit an IFN signature (Figure 2B).

Longitudinal analyses were performed on 29 SLE patients who had at least 3 visits (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Their median followup time was 8.3 months (range 2–28 months), with a median interval between visits of 3.2 months (range 0.5–19 months). Although module M1.2 was very stable over time within individual patients (coefficient of variation

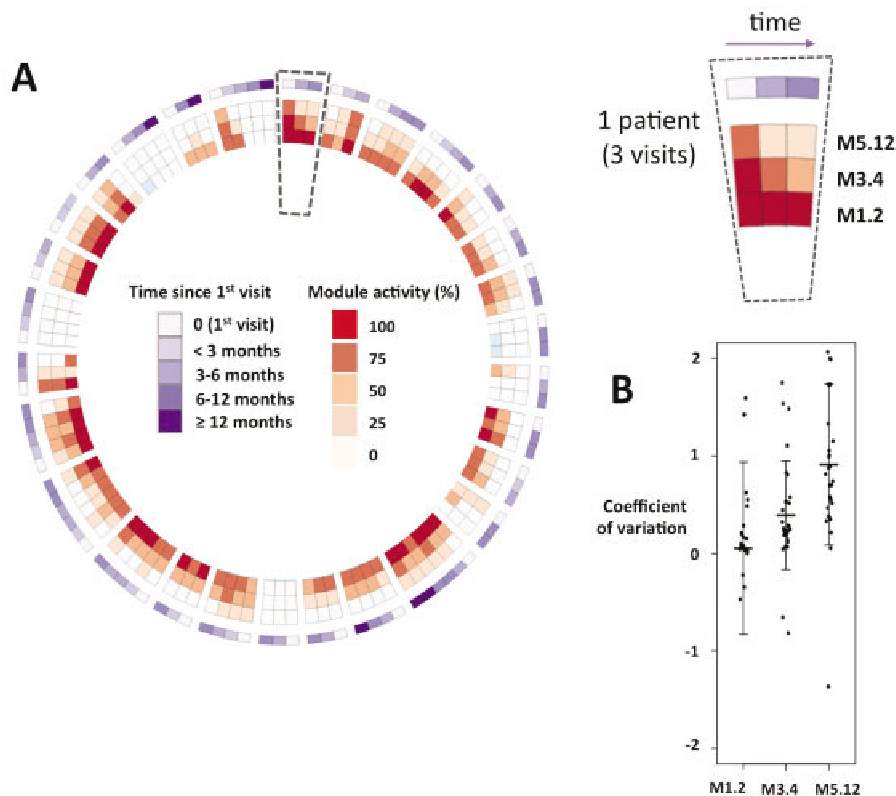

Figure 3. Longitudinal intraindividual variation of IFN modules in SLE patients. Longitudinal analyses were obtained for 29 SLE patients with at least 3 consecutive visits. **A,** The level of up-regulation of each IFN module at each visit is plotted on the Circos figure, representing, from the center to the periphery, M1.2, M3.4, M5.12, and time elapsed since the first visit. Spaces separate different patients (e.g., 3 visits by the same patient are framed by the dashed line). **B,** Mean ± SD coefficients of variation (CVs), corresponding to intraindividual variability of IFN modules, indicate that while M1.2 is stable over time for a given patient (CV 0.05 ± 0.88), M3.4 (CV 0.39 ± 0.56) and to a greater extent M5.12 (CV 0.91 ± 0.82) show fluctuations across time, reflecting the complexity of the IFN signature. These differences of variability between modules are significant (M1.2 CV versus M3.4 CV, $P = 0.0033$; M1.2 CV versus M5.12 CV, $P < 0.0001$; M3.4 CV versus M5.12 CV, $P = 0.00065$). See Figure 1 for other definitions.

[CV] 0.05), significantly much greater variation was seen for module M3.4 (CV 0.39) and especially module M5.12 (CV 0.91) (Figure 3), supporting the notion that a complex regulation of biologic pathways underlies the IFN signature of SLE, and that the 3 modules represent distinct IFN signatures.

Association of IFN modules and modular IFN score with disease activity in SLE patients. At the individual level, the levels of up-regulation of IFN modules (percentage difference between up-regulated and down-regulated probes in SLE patient samples versus control samples) were correlated with anti-dsDNA titers (Table 1). M1.2 levels did not correlate with the SELENA–SLEDAI score or with the presence of a flare (Table 1). Conversely, a weak but significant correlation was observed between expression of M3.4 and M5.12 modules and the presence of a flare, the expression of M5.12 being also correlated with the SELENA–SLEDAI score. Both M3.4 and M5.12 modules correlated with cutaneous flares, while only M5.12 correlated with renal flares (Table 1).

Individual clinical and biologic parameters were also compared at the group level according to modular IFN scores. No differences were observed concerning patient age, sex, ethnicity, or disease duration in the 4 groups (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Anti-dsDNA titers were significantly higher ($P = 0.03$) and lymphocyte counts were significantly lower ($P < 0.0001$) in patients with moderate or strong modular IFN scores compared to patients with absent or mild scores (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). The SELENA–SLEDAI score did not vary significantly between these groups, although a linear trend was observed ($P = 0.06$). Only cutaneous flares

Table 1. Correlations of IFN modules M1.2, M3.4, and M5.12 with clinical and biologic markers of SLE disease activity*

	M1.2	M3.4	M5.12
SELENA–SLEDAI score			
r	0.05	0.12	0.21
P	0.54	0.13	0.008
Flare (no flare, mild/moderate flare, or severe flare)			
r	0.12	0.2	0.28
P	0.13	0.012	0.0003
Cutaneous flare			
r	0.11	0.15	0.21
P	0.17	0.05	0.01
Articular flare			
r	−0.13	−0.024	0.023
P	0.11	0.77	0.77
Hematologic flare			
r	−0.012	−0.043	0.055
P	0.13	0.6	0.5
Renal flare			
r	0.066	0.13	0.22
P	0.41	0.1	0.007
Anti-dsDNA titer			
r	0.28	0.26	0.19
P	0.0007	0.0015	0.002
Low C3 or C4			
r	0.072	0.086	0.12
P	0.37	0.29	0.14

* The levels of up-regulation of interferon (IFN) modules (% difference) were correlated (Spearman's correlation coefficient [r]) with clinical and biologic markers of disease activity in the 157 samples from patients with systemic lupus erythematosus (SLE). SELENA–SLEDAI = Safety of Estrogens in Lupus Erythematosus National Assessment version of the SLE Disease Activity Index; anti-dsDNA = anti–double-stranded DNA.

were significantly more frequent in patients with moderate or strong modular IFN scores compared to patients with absent or mild scores (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Patients with moderate or strong modular IFN scores were less likely to receive antimalarial therapy ($P = 0.002$) or combined immunosuppressant and antimalarial therapies ($P = 0.0006$).

Modular IFN scores allow stratification of patients with clinically quiescent disease. Because IFN signatures can be rapidly influenced by recent treatment modifications (e.g., a severe flare and/or receiving high-dose corticosteroid pulses before sampling), the modular IFN signature was investigated in the group with clinically quiescent disease, which included 64 visits corresponding to 34 SLE patients (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). At the group level, the modular IFN signature was comparable to that of patients with active disease. At the individual level, 51 of 64 patients (80%) exhibited a modular IFN signature (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). No differences were observed concerning age, sex, ethnicity, or disease duration among these 34 patients with quiescent disease according to IFN score groups (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Anti-dsDNA titers were significantly higher ($P = 0.007$) and lymphocyte counts were significantly lower ($P < 0.0001$) in patients with moderate or strong modular IFN scores compared to patients with absent or mild scores (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). No significant differences in therapy were observed between patients with moderate or strong modular IFN scores and patients with absent or mild signatures.

Modular IFN signature and molecular insight into SLE pathogenesis. Variations in modular IFN signature across patients and within individual patients followed up longitudinally could reflect disease-specific pathogenic events, especially for those with clinically quiescent disease who had no modifications to their therapy. Specifically, the different IFN modular patterns could partially reflect the involvement of different types of IFNs. To test this hypothesis, we first analyzed the effects of exogenous IFN administration using publicly available data sets. We analyzed the effects of therapeutic IFNs on modular IFN signatures in patients treated with IFNα (for hepatitis C virus infection) (26) or with IFNβ (for multiple sclerosis) (25). Strikingly, administration of IFNα or IFNβ alone in these patients could not completely reproduce the modular IFN signature observed in SLE. Indeed, treatment with IFNα resulted in the up-regulation of M1.2 only, while treatment with IFNβ was associated in most patients with both a strong up-regulation of M1.2 and an up-regulation of M3.4. Conversely, transcripts belonging to M5.12 were poorly induced by IFNα or IFNβ alone (Figure 4A).

We also used the Interferome database to determine the extent to which genes belonging to IFN modules were inducible in vitro by the different IFNs. Genes significantly up-regulated in SLE patients compared to healthy controls were identified in each IFN-annotated module (27 genes in M1.2, 48 in M3.4, 48 in M5.12) and filtered to select genes registered as "interferon-related" (Figure 4B). Comparison of $\log_2$(fold change) after stimulation with different types of IFNs in this database showed that M1.2 transcripts were markedly induced more by type I IFN than by type II IFN ($P < 0.0001$). In addition, and consistent with what was observed with

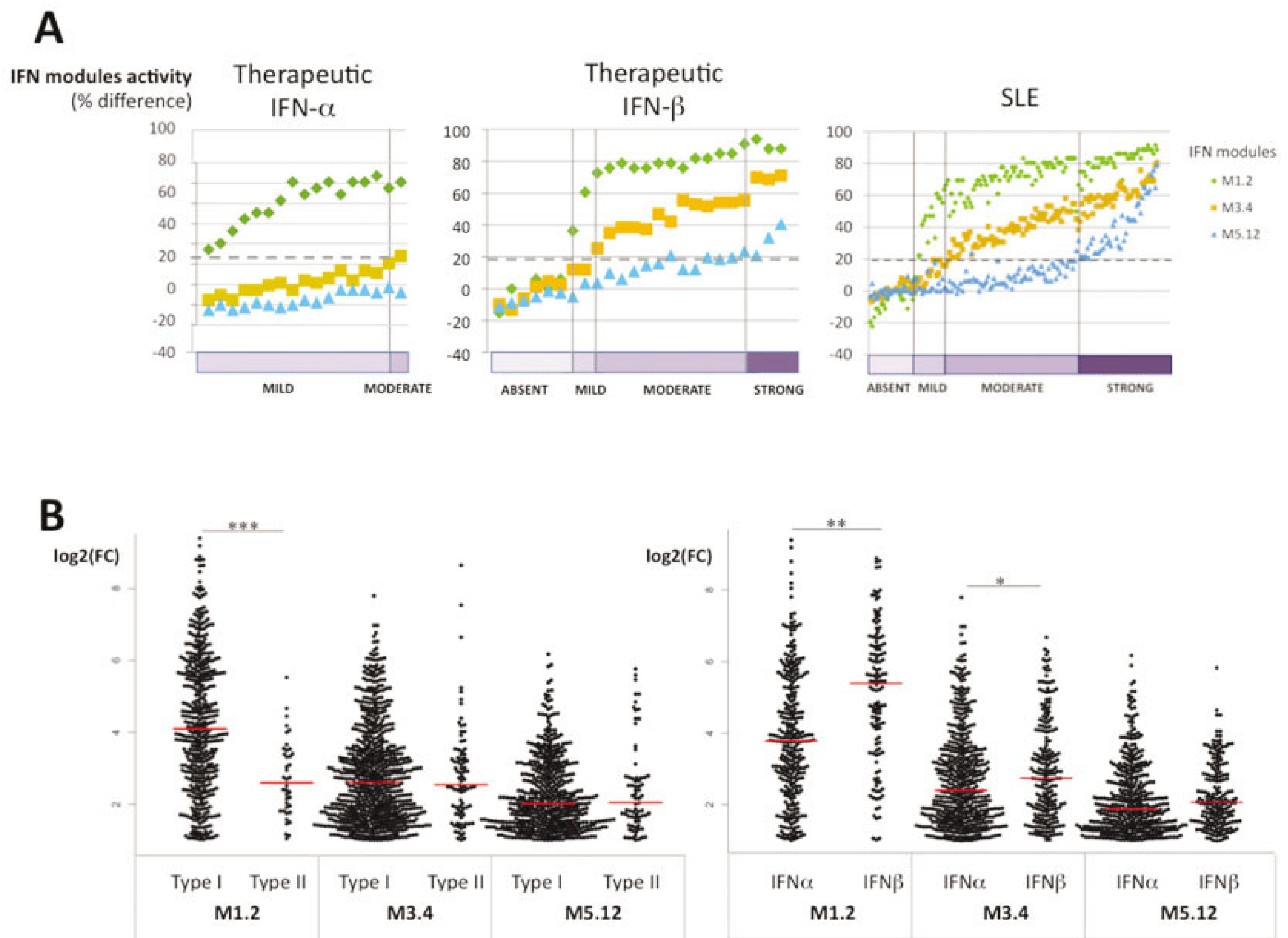

Figure 4. Effect of different types of IFN on IFN modular patterns. Modular analysis was performed on gene expression data from 2 public domain data sets (25,26). **A,** At the individual level, treatment with IFNα results in the up-regulation of M1.2 only, while treatment with IFNβ is associated with up-regulation of both M1.2 and M3.4 in most patients, as well as with up-regulation of M5.12 in some patients. The graph at right represents the modular analysis obtained in SLE patients from the LUPUCE cohort. Horizontal dashed lines correspond to the 20% difference in regulation (% up-regulated probes − % down-regulated probes) above which a module was considered "active." Symbols represent individual samples. **B,** The responsiveness to different types of IFN of the genes from the 3 IFN modules was evaluated using the Interferome database. The $\log_2$(fold change [FC]) observed in each experiment for each gene after in vitro stimulation was compared between type I and type II IFNs, as well as between IFNα and IFNβ, and is represented on the bee-swarm plots. Symbols represent individual transcripts; horizontal lines show the median. Transcripts belonging to M1.2 are induced significantly more by type I IFN than by type II IFN (median $\log_2$[fold change] 4.10 versus 2.60; $*** = P < 0.0001$), while transcripts belonging to M3.4 and M5.12 are similarly induced by type I and type II IFNs (2.60 versus 2.54; $P = 0.87$ and 2.01 versus 2.04; $P = 0.68$, respectively). In addition, M1.2 and M3.4 transcripts are induced significantly more by IFNβ than by IFNα (5.39 versus 3.78; $** = P = 0.0001$ and 2.74 versus 2.40; $* = P = 0.034$, respectively), while transcripts belonging to M5.12 only exhibit a nonsignificant trend in favor of IFNβ (2.07 versus 1.89; $P = 0.051$). See Figure 1 for other definitions.

in vivo data, M1.2 and M3.4 transcripts were induced significantly more by IFNβ than by IFNα ($P = 0.0001$ and $P = 0.034$, respectively). Finally, transcripts belonging to M3.4 and M5.12 were similarly induced in vitro by type I and type II IFNs. Overall, these results suggest that the dynamic modular IFN signature observed in SLE patients is not exclusively driven by IFNα; IFNβ could play a role in the up-regulation of these modules, and type II IFN, in combination with type I IFNs, could contribute to the full-blown response of M3.4 and M5.12.

We next used a gene-level whole-transcriptome approach to characterize differences between modular IFN score groups in patients with clinically quiescent

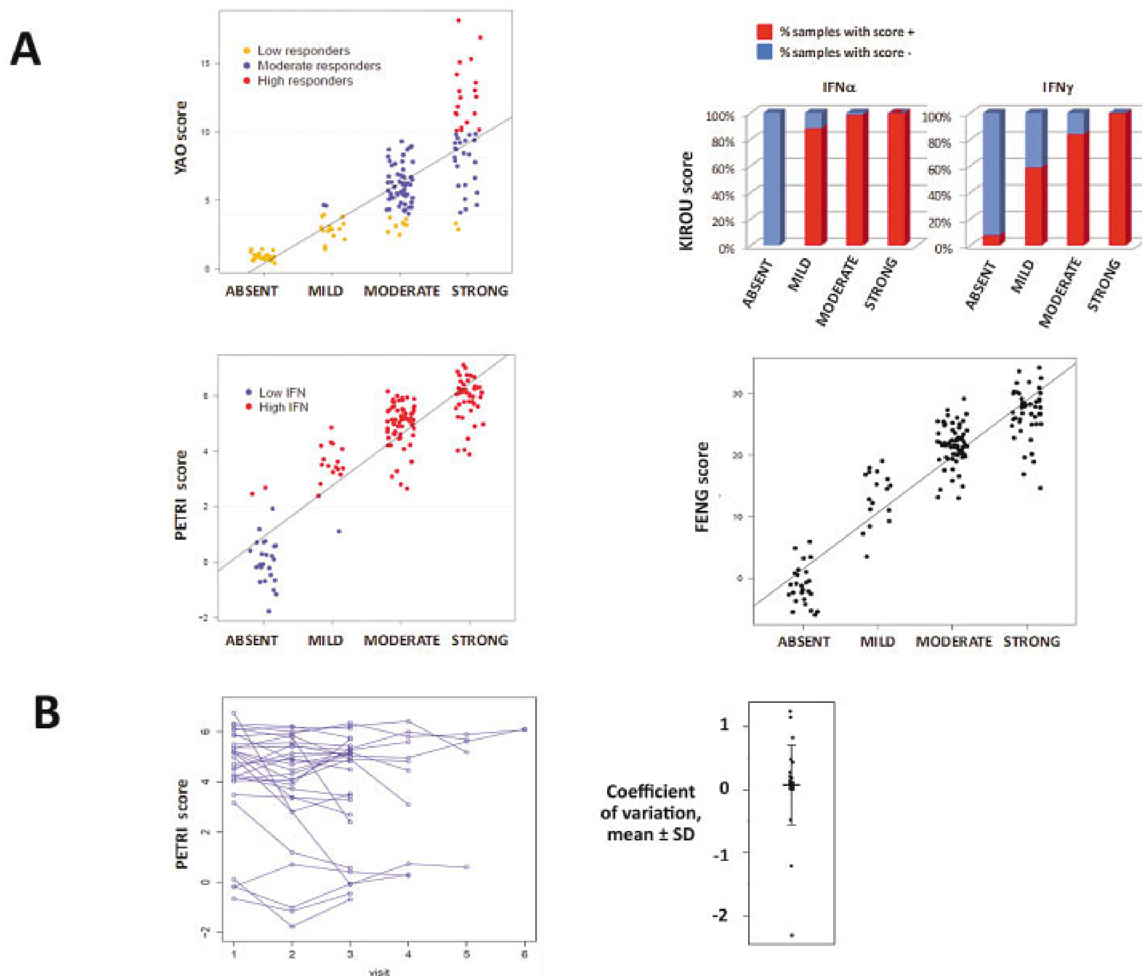

Figure 5. Interscore correlations in SLE patients. **A,** The modular IFN score (absent/mild/moderate/strong) is compared to 4 scores from the literature using the 157 samples from SLE patients in the present cohort. The linear model shows an increase in the Yao score (28) of 2.9 points per category of modular IFN score ($P < 0.0001$), an increase in the Petri score (8) of 1.85 points per category ($P < 0.0001$), and an increase in the Feng score (29) of 9.14 points per category ($P < 0.0001$). Both IFNα and IFNγ Kirou scores (30) were linked with the modular IFN score ($P < 0.0001$ by chi-square test for trend in proportions). **B,** Shown is the Petri IFN score across time in samples from 29 patients with ≥3 visits (the "longitudinal" group). A limited interindividual variability of the Petri IFN score across time is observed (mean of the patient coefficients of variation 0.07). This score is based on the expression of 3 IFN-inducible genes (IFI27, IFI44, and OAS3). Symbols represent individual samples. See Figure 1 for definitions.

disease. There were 209 transcripts that were differentially expressed in these patients compared to healthy controls; these corresponded to 171 unique gene symbols, of which 75 were registered as "IFN-related" in Interferome. Corresponding transcripts were used to calculate an "IFN molecular distance to health" for each sample; this was strongly correlated to modular IFN score ($r = 0.946$, $P < 0.0001$) (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Samples from SLE patients with quiescent disease were listed according to their modular IFN score, and a hierarchical clustering of the 209 differentially expressed transcripts was performed. Five clusters were identified (genes and detailed pathway annotations are available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Three of them (clusters 3, 4, and 5) were annotated as "IFN-related" but exhibited various patterns of expression in the different modular IFN score groups. Cluster 4, "IFN early signaling–related," was up-regulated similarly in patients with mild, moderate, or strong modular IFN scores (but not in patients with

absent scores); up-regulation of transcripts belonging to clusters 3 and 5 only occurred in patients with moderate and strong scores. Interestingly, cluster 3 was annotated as "IFN-regulated chemotaxis" and cluster 5 was annotated as "IFN downstream signaling–related," which suggests that the modular IFN score could reflect sequential pathogenic events that occur from a state of immunologic quiescence (absent modular signature) to dynamic activation of transcripts from the early to downstream parts of the IFN pathway.

Modular analysis: a new approach for longitudinal analyses and biomarker development in SLE? For clinical applicability, an approximation of modular IFN score should be obtainable using focused assays such as PCR and targeting only a few genes. First, to validate the microarray data, we confirmed the overexpression of 16 genes belonging to the 3 IFN modules using whole-blood quantitative reverse transcription–PCR in the same SLE patient samples (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). There were excellent correlations between microarray and TaqMan assays across samples (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). We also used a random forest approach to reduce this to the best 3 classifiers of each of the modular IFN signatures. We could accurately classify samples according to the predefined categories of modular IFN score (absent/mild versus moderate/strong) with an average overall error of 4.7% (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_supplemental_data.pdf).

We compared our modular IFN signatures to other previously established IFN scores from the literature. Although all scores were strongly correlated with the modular IFN signature (Figure 5A), they were not redundant. For example, although most samples were considered "high IFN" using the Petri IFN score, these samples could belong to mild, moderate, or strong groups according to our modular IFN scores. Similarly, patients considered "low responders" using the Yao IFN score could belong to different groups according to our modular IFN score (mostly absent or mild, but also moderate or strong). Interestingly, all patients with an "absent" modular IFN score had a negative IFNα Kirou score, while all patients with a strong modular IFN score had positive IFNα and IFNγ Kirou scores (Figure 5A), which is consistent with the possible implication of type II IFN in the activity of modules M3.4 and M5.12. Concerning longitudinal variations in IFN signatures, there was no significant variation in the Petri IFN score across time in our samples (mean of the patient CVs 0.07) (Figure 5B), while a variation was identified using the modular IFN score (Figure 3). Notably, genes selected for IFN scores according to the literature are mostly from M1.2 (the least variable module) and to a lesser extent M3.4, but very few belong to M5.12 (the more variable IFN module) (further information is available at http://fr.ap-hm.fr/sites/default/files/lupuce_study_ supplemental_data.pdf).

DISCUSSION

Microarray technology is a valuable tool for gaining insight into the molecular complexity of autoimmune diseases and for identifying new therapeutic targets and biomarkers (15). Just a decade ago, the first SLE transcriptome studies revealed a blood IFNα signature (32,33). These data were supported by many additional studies (8,9,17,29,30,34,35) and led to the development of IFNα blockers that are currently being tested in clinical trials (7).

Here we report original results from data-driven constructed framework models of gene sets (16) to assess, cross-sectionally and longitudinally, the blood transcriptional profiles of adult SLE patients. This modular approach has confirmed the high prevalence of IFN signatures in these patients, as reported using various IFN scoring systems (5,6). Strikingly, however, the IFN signature was more complex than expected; a dynamic modular IFN signature was observed in SLE patients, with each component module displaying a distinct activation threshold. Corresponding transcripts, reflecting distinct aspects of the IFN signaling pathway, were not exclusively IFNα inducible, but might also have been induced by IFNβ and IFNγ. In addition, the modular IFN signature correlated with serologic disease activity (anti-dsDNA titer and lymphocyte count) and, to some extent, with clinical activity. Interestingly, this modular signature was variable across time in single individuals; M1.2 was stable, while M3.4 and M5.12 did vary. Importantly, the modular signature allowed stratification of patients with quiescent disease, and we observed correlations of the modular score with SLE biologic activity parameters as well as with intensity of maintenance treatments. Additional studies are therefore needed to test the usefulness of the IFN modular score to predict future flares in these SLE patients with clinically quiescent disease.

These results differ from those recently reported, which show no significant variation in IFN scores across time and no correlations with disease activity (8,9). In-

terestingly, most reported transcripts selected to build IFN scores belong to M1.2, the least variable of the IFN modules (further information is available at http://fr.aphm.fr/sites/default/files/lupuce_study_supplemental_data.pdf). Because therapies for flares, especially high-dose corticosteroids, can rapidly "extinguish" the IFN signature (36), we repeated our analyses on patients with quiescent disease and no recent treatment modifications and were able to show the same variability. Although our modular IFN score was strongly correlated with previously reported IFN scores, it differed inherently from them, perhaps allowing a more global and qualitative overview of IFN pathway activation. Thus, a modular approach that relies not only on knowledge (choice of genes previously identified as IFN inducible) and/or expression intensity (genes with the highest fold changes) but also on hypothesis-free and data-driven descriptions could result in a "qualitative" appreciation of the IFN signature in individual SLE patients and aid the construction of new SLE biomarkers.

Having observed this dynamic IFN signature in SLE, we used the modular IFN score to stratify patients and compare gene expression across these groups. Indeed, combining our modular approach with the Interferome database, we conclude that not only IFNα, but also IFNβ and IFNγ may contribute to the IFN signature in SLE. Indeed, as recently stated by others in the context of infectious diseases (37–39) and autoimmune systemic diseases (11,12), there is a large overlap between type I IFN– and type II IFN–inducible signatures. Interestingly, the transcripts selected by Hall et al (12) to discriminate between type I IFNs (IFN-induced protein with tetratricopeptide repeats 3 [IFIT-3] and melanoma differentiation–associated protein 5 [MDA-5]) and type II IFNs (guanylate binding protein 1 [GBP-1] and GBP-2) in tissue biopsy samples from patients with Sjögren's syndrome reflect the type I–to–type II transition observed between our 3 IFN modules; IFIT-3 belongs to M1.2, MDA-5 and GBP-1 belong to M3.4, and GBP-2 belongs to M5.12. Also, IFN-inducible chemokines (CCL2, CXCL10, CCL19) and B lymphocyte stimulator/BAFF, whose circulating levels have been associated with disease activity (15,40), are both type I and type II IFN inducible (13).

Finally, recent reports of overlapping downstream signaling events (1,14) demonstrate cross-talk between type I and type II IFN signaling pathways. Some experiments support the role of low-dose type I IFNs in priming cells to secrete type II IFNs (41). Similarly, the ability of IFNγ to enhance signaling through Toll-like receptors may enhance type I IFN secretion (42). Studying gene expression in the context of viral infection, Su et al have demonstrated that type I IFN–induced genes dominate the early phase of viral infection, whereas IFNγ (triggered by IFNα) and its targets were responsible for the effector phase of the antiviral responses (43). Our results suggest a dynamic IFN response in SLE, driven by the interaction of genes induced by various IFNs. The theory of a sequential role of type II IFN following type I IFNs is supported by murine data on the development of lupus nephritis (44,45) and was previously suggested by Crow et al (46,47).

We show that only the third IFN module, M5.12, correlated with renal flares in our cohort. Disappointing results for IFNα blockade have been reported recently in clinical trials in SLE and psoriasis (48–50). In a phase II clinical trial in SLE patients, a significant response was observed only in patients with a "low-level" IFN signature (49). These surprising results may suggest a need for stronger inhibition of IFNα in patients with a "high-level" signature (7), or they may indicate that IFNs other than IFNα are involved in SLE disease pathogenesis and that targeting IFNα alone in SLE may not be sufficient to control disease activity.

This study has several limitations. First, whole-blood gene expression analysis does not permit the identification of cell-specific components of the IFN signature (51). However, it allows easy sample collection and preparation, which are fundamental elements for clinical applicability. Second, modular IFN signatures may differ according to ethnic backgrounds, and most patients in our cohort were Caucasian. However, we observed similar patterns in the pediatric cohort described by Berry et al (22), which contained mostly Hispanic and African American patients (only 15% non-Hispanic white patients), and in the adult cohort described by Arasappan et al (24), which included only 44% white patients. Finally, the dynamic modular signature we observed could have been linked to the use of SLE patient samples, as SLE was included in the building of the original modular framework (17). However, the different IFN signature pattern observed in samples from patients with tuberculosis, which was also used in the original framework construction, rules out this possibility and instead supports the diversity of IFN pathways in different human diseases.

Taken together, the results obtained using our modular framework support the hypothesis that the IFN signature observed in SLE patients corresponds to more IFN types than just IFNα. This approach may enable a better molecular stratification of SLE patients. Ulti-

mately, these modular signatures may aid in the design of biomarkers to assess disease activity and in the selection and monitoring of therapies for SLE as well as other possible IFN-related autoimmune (34) or infectious (52) conditions.

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All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Chiche had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study conception and design. Chiche, Jourde-Chiche, Burtey, Berland, Harle, Pascual, Chaussabel.
Acquisition of data. Chiche, Jourde-Chiche, Gersuk, Anguiano, Burtey, Berland, Kaplanski, Chaussabel.
Analysis and interpretation of data. Chiche, Jourde-Chiche, Whalen, Presnell, Gersuk, Dang, Quinn, Berland, Pascual, Chaussabel.

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