An Interferon Signature in the Peripheral Blood of Dermatomyositis Patients is Associated with Disease Activity

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Recent studies have shown increased expression of interferon (IFN)-regulated genes in the peripheral blood cells of patients with systemic lupus erythematosus. A similar interferon signature has been observed in affected muscle tissue from patients with dermatomyositis (DM), but it has not yet been determined if this signature extends to the peripheral blood in DM. We performed global gene expression profiling of peripheral blood cells from adult and juvenile DM patients and healthy controls. Several interesting groups of genes were differentially expressed in DM, including genes with immune function, and others that function in muscle or are involved in mitochondrial/oxidative phosphorylation. Investigation of type I IFN-regulated transcripts revealed a striking interferon signature present in most DM patients studied. Levels of type I IFN-regulated proteins were also elevated in DM serum samples. Furthermore, both the transcript and serum protein IFN signatures were associated with disease activity. These data suggest that the IFN signature may be a useful marker for DM disease activity, and that sampling peripheral blood may be a more practical alternative to muscle biopsy for measuring this signature.

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INTRODUCTION

Juvenile and adult dermatomyositis (DM) are rare autoimmune diseases belonging to the group of idiopathic inflammatory myopathies (IIM), which are characterized by proximal muscle weakness and muscle inflammation. In cases of adult and juvenile DM, patients also present with a characteristic skin rash (1). Juvenile dermatomyositis is the most common pediatric IIM. Although the cause of IIM is unknown, it is believed that a combination of genetic and environmental factors contribute to disease. Juvenile DM is known to be associated with the HLA class II region genes HLA-DQA1*0501, and polymorphisms in TNFα are associated with disease severity (2). In both adult and juvenile DM, associations exist with DR3 (2,3). Current evidence suggests that the vasculature and perifascicular regions in muscle are targets for both humoral- and cell-mediated autoimmunity. It has been proposed that viral infection is a trigger for initiation of juvenile DM (4), but this has not yet been convincingly demonstrated.

Several studies have recently reported the results of gene expression profiling from muscle biopsies of patients with adult and juvenile DM (5–9). The emerging data suggest that cells from affected muscle tissue carry distinct immune fingerprints which may be useful for monitoring disease status and may provide new targets for disease modifying agents. Two of these studies reported up-regulation of genes related to antigen processing (7,8). Immunoglobulin transcripts, particularly those encoding Igλ light chains and IgG heavy chains, were also found at elevated levels in two separate studies (7,9).

Interestingly, interferon (IFN) pathway activation was observed in all reported studies and was largely specific to DM as opposed to other types of IIM (5,7–9). Greenberg et al. further documented the presence of an IFN-inducible protein, MxA, in muscle fibers and capillaries of DM patients (5).

Recently, much attention has been focused on type I IFN for its potential role in the autoimmune disease systemic lupus erythematosus (SLE) (10,11). Peripheral blood cells from SLE patients exhibit a striking pattern of up-regulation of IFN-induced genes (12–14). Approximately 50-75% of adult patients carry the IFN signature, and it is strongly correlated with disease activity and certain clinical manifestations of SLE, including anti-dsDNA antibodies and low complements (15,16). Interestingly, evidence for IFN pathway activation is also found in individual glomeruli isolated from

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the kidneys of patients with lupus nephritis (17). Plasmacytoid dendritic cells (PDC), the primary producers of type I IFN, are recruited to inflamed lupus skin lesions (18,19) and are also found in substantial numbers in affected adult and juvenile DM muscle tissue (5). IFN signatures have also been observed in Sjögren's syndrome and psoriasis, but not in rheumatoid arthritis or multiple sclerosis (20).

Although several interesting signatures have been reported in muscle tissue from adult and juvenile DM, it has not yet been determined whether these or other signatures are present in the blood. To address this, we examined global gene expression patterns in peripheral blood cells of DM patients and healthy controls using Affymetrix GeneChips. Several interesting groups of differentially expressed transcripts were observed in DM, including genes that function in muscle, and others involved in mitochondrial/oxidative phosphorylation and immune function. We also identified a prominent type I IFN gene and protein signature in most of the adult and juvenile DM patients studied, which correlated with disease activity. These data suggest that the IFN signature may serve as a marker for DM disease activity in adult and juvenile subjects, and that identification of the signature in peripheral blood samples is an alternative to the more invasive technique of muscle biopsy.

MATERIALS AND METHODS

Study Participants and Sample Collection

The study protocol was approved by the Human Subjects Institutional Review Boards at the University of Minnesota Medical School, the Mayo Clinic, and the Johns Hopkins School of Medicine, and informed consent was obtained from each participant. DM patients (n = 10) and JDM patients (n = 2) were evaluated in the Rheumatology Division at the Mayo Clinic in Rochester, MN and all met Bohan and Peter criteria for DM or JDM (21,22). Clinical data were collected for each patient, including duration of disease (average 23 ± 25 months, range 1–84 months), muscle biopsy (available for eight patients), and medications (11 patients on oral prednisone, average 20 ± 19 mg/day; three patients on hydroxychloroquine; eight patients on immunosuppressive therapy, including methotrexate, azathioprine, or mycophenolate mofetil). Standard clinical lab tests were also performed, in addition to measurement of autoantibodies and specific enzymes (creatine kinase, or CK; aldolase; aspartate aminotransferase, or AST; and alanine aminotransferase, or ALT). One patient exhibited unusually high levels of these enzymes; for purposes of analysis, these outlier values were truncated to a clinically relevant maximum (CK, 500 units/L; aldolase, 15 units/L; AST, 100 units/L; ALT, 75 units/L). Four patients had measurable autoantibodies (one positive for ANA; one positive for anti-Jo1; one positive for anti-SSA and anti-RNP; one positive for ANA, anti-SSA, and anti-Jo1). Current disease activity was evaluated using recently published measures (23,24), including the childhood myositis assessment scale (CMAS, used in the JDM patients), the (childhood) health assessment questionnaires (HAQ/CHAQ), manual muscle testing, elevation of muscle enzymes, and the extramuscular activity measure. Disease activity was given a rating of 0–2 based on the combination of these measures.

Biological samples collected at each visit included RNA from whole blood (using the PAXgene system from Qiagen/Becton-Dickinson), DNA, serum, plasma, and peripheral blood mononuclear cells (cryopreserved). The adult and pediatric DM patient group was 91% Caucasian and 73% female, with an average age of 43 ± 26 years. The matched control group (n = 15) was 89% Caucasian and 74% female, with an average age of 40 ± 10 years. SLE patients (n = 73) were enrolled from the Hopkins Lupus Cohort (25) as part of the Autoimmune Biomarkers Collaborative Network (ABCoN). Overall the SLE group had mild/moderate disease activity on the day of the blood draw (SLE disease activity index range 0-10). Current symptoms/organ involvement included rash (25% of patients), arthritis (30%), renal disease (18%), and hematologic manifestations (18%). Medications included oral prednisone (52% of patients; average 8.8 ± 6.8 mg/day), hydroxychloroquine (63% of patients), and immunosuppressive drugs (37% of patients).

Sample Processing for Microarray Analysis

For RNA isolation, blood was drawn into PAXgene tubes (PreAnalytix, Franklin Lakes, NJ). Total RNA was isolated according to the manufacturer's protocol with on-column DNAse treatment. RNA yield and integrity were assessed using an Agilent Lab-on-a-Chip Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA). cRNA probes were generated using the MessageAmp cRNA kit (Ambion, Austin, TX) and hybridized to Affymetrix U133 2.0 + GeneChips according to standard Affymetrix protocols (Expression Analysis Technical Manual, Affymetrix, Santa Clara, CA). After scanning the arrays, Expressionist Refiner (GeneData, Basel, Switzerland) was used to generate expression (or "signal") values for each gene using the Affymetrix Microarray Suite 5.0 condenser and scaling the overall intensity of each chip to 1500. All chips passed both the Microarray Suite and Refiner quality control measures.

Microarray Data Analysis

Uncharacterized genes were removed from the dataset before performing the analysis, leaving 38,059 probe sets. Genes identified as differentially expressed between DM and healthy controls met all three of the following criteria: (1) p-value of less than 0.0005 by unpaired t-test, (2) fold-change of at least 1.5 between the mean expression in DM and the mean expression in controls, and (3) absolute difference of at least 200 signal units between the mean expression in DM and

the mean expression in controls. A list of 665 unique genes met these filtering criteria. When correction for multiple testing was applied to the entire dataset (38,059 probe sets) using the method of Benjamini and Hochberg (26), the p-values for these 665 genes remained significant (adjusted $P < 0.05$). Expression data for these 665 genes are available in Supplementary Table 1 available on the Molecular Medicine Website (www.molmed.org). As an independent method of gene selection, Significance Analysis of Microarrays (SAM) was also applied to the dataset (27). Of the 665 DM genes identified by our criteria, 87% were also called significant by SAM, which identified a total of 686 differentially expressed genes ($\delta = 2.35$, fold change ≥ 1.5). Hierarchical clustering was performed using Cluster and visualized with Treeview (28). To improve visualization, each datapoint was transformed prior to clustering into a $\log_2$ ratio relative to the mean expression of normal controls. For identification of the IFN signature, we used a set of 315 genes previously identified as IFN-regulated in control blood cells after 6 h of treatment in vitro with either IFN-α/β or IFN-γ (12). The DM and control expression data for these IFN-regulated genes were subjected to hierarchical clustering. IFN signature scores were calculated using a subset of 43 IFN-regulated genes that were found in both the DM IFN signature and the SLE IFN signature (SLE data unpublished). To calculate IFN scores, the expression data were normalized so that the maximum value of each gene was 1.0. The normalized expression values of the 43 genes were then summed up to obtain the IFN score. Expression data for these 43 genes are available in Supplementary Table 2 available on the Molecular Medicine Website (www.molmed.org).

Serum Protein Measurement

Serum was isolated from blood drawn into serum-separator vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ). A protease inhibitor (aprotinin, 1μg/mL) was added to each sample, and aliquots were immediately frozen at –80°C. Serum was available from 10 of the 12 DM patients (nine adults and one juvenile) analyzed by microarray. The levels of 12 serum proteins were measured using Protein Multiplex Immunoassays (Biosource, Carlsbad, CA: IP-10, MIG, MCP-2, TNF-RI; R&D Systems, Minneapolis, MN: ENA-78, IL-6, IL-8, I-TAC, MCP-1, MIP-1A, MIP-1B, MMP-7) coupled with Luminex xMAP technology. Samples were run in duplicate, and calibrated recombinant proteins were used to generate standard curves.

RESULTS

Gene Expression Profiles Distinguish DM Patients from Healthy Controls

Peripheral blood gene expression profiling was used to identify molecular fingerprints of patients with adult and juvenile DM. Comparison of whole blood gene expression profiles between DM patients and healthy, matched controls revealed a set of 665 unique transcripts that were found at significantly altered levels in DM ($P < 0.0005$ by unpaired t-test, average fold change > 1.5, and average difference in expression > 200 units). Hierarchical clustering of these genes clearly distinguished the patients from the controls (Figure 1). The majority of the differentially expressed genes were found at lower levels in DM patients. The adult and juvenile patients did not cluster separately from one another.

Functional characterization of genes that were differentially expressed in DM revealed several interesting groups of transcripts (Table 1). These included a set of immune-related genes, with transcripts encoding several cytokines and receptors. Several immune signaling molecules were down-regulated in DM, including the tyrosine kinase FYN, inducible T-cell co-stimulator ligand (ICOSL), and several MAP kinase family members. NFATC4, a member of the nuclear factors of activated T cells DNA-binding transcription complex, was found at elevated levels in DM blood cells. The inflammatory mediator S100A8 and B cell activating factor TNFSF13B (BAFF) were also up-regulated in DM. Of note, several natural killer (NK) cell receptors were found at decreased levels in DM. These included killer cell immunoglobulin-like receptors (KIRs), which are MHC class I receptors found on NK cells. Another type of NK cell receptor, a killer cell lectin-like receptor (KLRG1), was also down-regulated in patients. Furthermore, several transcripts encoding autoantigens were found at altered levels in DM. Decreased expression of heterogeneous nuclear ribonucleoproteins (hnRNPs) was observed, while genes encoding histones, autoantigens commonly targeted in Sjogren's syndrome and scleroderma (SSSCA1), and the nuclear antigen SP100 were up-regulated. Several muscle-related transcripts were also differentially expressed in DM. These include genes encoding myosin and related proteins, desmin, and several collagen subunits.

Many of the genes differentially expressed in DM are involved in oxidative pathways or other mitochondrial functions. Some components of the electron transport chain, such as cytochrome C oxidase and NADH dehydrogenase, were up-regulated in DM, while a subunit of the mitochondrial ATPase (ATP5G2) was down-regulated. Several members of the cytochrome p450 family of oxidative enzymes were found at decreased levels in DM. The antioxidants thioredoxin and peroxiredoxin five were up-regulated in patients, while glutathione peroxidase was down-regulated.

Blood Cells of DM Patients Exhibit an IFN Gene Expression Signature

Despite published reports of IFN pathway activation in DM muscle tissue (5, 7–9), IFN-regulated genes were not prominent among the most differentially expressed transcripts between DM patients and healthy controls. To look for evidence of IFN pathway dysregulation in DM, we examined the expression levels of 315 genes previ-

Table 1. Selected genes differentially expressed in DM are grouped according to functional classification. The average fold change in DM compared with healthy controls is included ("Avg FC"), in addition to the p-value from unpaired t-test.

	Entrez Gene	Gene Title	Gene Symbol	Avg FC	p-value
Immune-related	27178	interleukin 1 family, member 7 (zeta)	IL1F7	–2.1	4.5×10^{-5}
	54756	interleukin 17 receptor D	IL17RD	–2.1	4.1×10^{-4}
	50604	interleukin 20	IL20	–2.1	2.7×10^{-4}
	282618	interleukin 29 (interferon, lambda 1)	IL29	–1.8	4.4×10^{-4}
	3656	interleukin-1 receptor-associated kinase 2	IRAK2	–4.7	5.9×10^{-6}
	2833	chemokine (C-X-C motif) receptor 3	CXCR3	–2.5	4.2×10^{-4}
	975	CD81 antigen	CD81	–3.8	7.8×10^{-6}
	6279	S100 calcium binding protein A8 (calgranulin A)	S100A8	3.5	2.5×10^{-4}
	10673	TNF ligand superfamily, member 13b (BAFF)	TNFSF13B	2.9	1.4×10^{-4}
	2534	FYN oncogene related to SRC, FGR, YES	FYN	–1.6	2.4×10^{-4}
	23308	inducible T-cell co-stimulator ligand	ICOSLG	–1.9	2.4×10^{-4}
	4776	nuclear factor of activated T-cells, cytoplasmic 4	NFATC4	3.0	4.9×10^{-4}
	79109	MAP kinase associated protein 1	MAPKAP1	–2.4	9.7×10^{-7}
	6885	MAP kinase kinase kinase 7	MAP3K7	–1.6	1.8×10^{-4}
	4293	MAP kinase kinase kinase 9	MAP3K9	–1.9	1.8×10^{-5}
	3811	killer cell Ig-like receptor, 3 domains, long 1	KIR3DL1	–2.8	3.5×10^{-5}
	3803	killer cell Ig-like receptor, 2 domains, long 2	KIR2DL2	–4.6	6.1×10^{-8}
	3804	killer cell Ig-like receptor, 2 domains, long 3	KIR2DL3	–2.1	4.6×10^{-6}
	57292	killer cell Ig-like receptor, 2 domains, long 5A	KIR2DL5A	–3.4	2.0×10^{-7}
	3809	killer cell Ig-like receptor, 2 domains, short 4	KIR2DS4	–4.4	3.6×10^{-4}
	3810	killer cell Ig-like receptor, 2 domains, short 5	KIR2DS5	–3.2	7.9×10^{-6}
	10219	killer cell lectin-like receptor subfamily G 1	KLRG1	–1.6	2.6×10^{-5}
Autoantigens	120364	heterogeneous nuclear ribonucleoprotein A1	HNRPA1	–2.2	8.8×10^{-6}
	3183	heterogeneous nuclear ribonucleoprotein C (C1/C2)	HNRPC	–1.9	1.8×10^{-4}
	9987	heterogeneous nuclear ribonucleoprotein D-like	HNRPDL	–2.0	7.5×10^{-5}
	3192	heterogeneous nuclear ribonucleoprotein U	HNRPU	–1.6	2.4×10^{-4}
	8331	histone 1, H2aj	HIST1H2AJ	2.1	3.9×10^{-4}
	8337	histone 2, H2aa	HIST2H2AA	2.3	1.3×10^{-4}
	10534	Sjogren's syndrome/scleroderma autoantigen 1	SSSCA1	2.0	5.2×10^{-5}
	6672	nuclear antigen Sp100	SP100	1.6	3.7×10^{-4}
Muscle-related	23026	myosin heavy chain Myr 8	MYR8	–2.5	7.4×10^{-5}
	29116	myosin regulatory light chain interacting protein	MYLIP	–2.0	4.4×10^{-5}
	10627	myosin regulatory light chain MRCL3	MRCL3	1.7	4.6×10^{-4}
	55930	myosin VC	MYO5C	–1.6	4.7×10^{-4}
	8082	sarcospan (Kras oncogene-associated gene)	SSPN	–1.8	3.4×10^{-4}
	81493	syncoilin, intermediate filament 1	SYNC1	–2.0	3.8×10^{-4}
	1285	collagen, type IV, α 3 (Goodpasture antigen)	COL4A3	–3.0	2.2×10^{-4}
	1287	collagen, type IV, α 5 (Alport syndrome)	COL4A5	–2.4	3.2×10^{-4}
	1288	collagen, type IV, α 6	COL4A6	–3.2	1.8×10^{-5}
	1291	collagen, type VI, α 1	COL6A1	–2.8	1.4×10^{-5}
	1674	desmin	DES	4.2	2.7×10^{-4}
Mitochondrial/oxidative	1329	cytochrome c oxidase subunit Vb	COX5B	5.7	1.3×10^{-4}
	1337	cytochrome c oxidase subunit VIa polypeptide 1	COX6A1	1.9	6.8×10^{-5}
	1353	COX11 homolog	COX11	–2.1	4.9×10^{-4}
	517	ATP synthase, mitochondrial F0 complex, c 2	ATP5G2	–4.8	2.8×10^{-5}
	93974	ATPase inhibitory factor 1	ATPIF1	1.6	3.0×10^{-5}
	4695	NADH dehydrogenase 1 α 2	NDUFA2	1.5	3.1×10^{-4}
	4710	NADH dehydrogenase 1 β 4	NDUFB4	1.5	1.6×10^{-4}
	5832	aldehyde dehydrogenase 18 family, member A1	ALDH18A1	–1.8	1.1×10^{-5}
	221	aldehyde dehydrogenase 3 family, member B1	ALDH3B1	–3.4	3.6×10^{-5}
	1544	cytochrome P450, family 1 A2	CYP1A2	–1.7	1.8×10^{-4}
	1584	cytochrome P450, family 11 B1	CYP11B1	–2.7	3.4×10^{-5}
	1559	cytochrome P450, family 2 C9	CYP2C9	–3.0	1.3×10^{-5}
	1579	cytochrome P450, family 4 A11	CYP4A11	–2.8	1.3×10^{-4}
	1595	cytochrome P450, family 51 A1	CYP51A1	–1.6	2.7×10^{-4}
	1582	cytochrome P450, family 8 B1	CYP8B1	–2.1	1.5×10^{-4}
	2878	glutathione peroxidase 3 (plasma)	GPX3	–2.0	1.6×10^{-5}
	25824	peroxiredoxin 5	PRDX5	1.7	2.4×10^{-4}
	7295	thioredoxin	TXN	2.8	1.1×10^{-6}
	65003	mitochondrial ribosomal protein L11	MRPL11	1.6	2.0×10^{-4}

ously identified as type I IFN-regulated (12). Hierarchical clustering of the expression data for these genes in the patient and control samples revealed that a subset of IFN-regulated genes are indeed up-regulated in the blood cells of most, but not all, DM patients (Figure 2). Many of these genes also belong to the SLE IFN signature (Figure 2, green bars). Ten of the twelve patients (nine adults and one juvenile) and one control formed a cluster of samples showing up-regulation of IFN-induced genes. In contrast, two DM patients (one adult and one juvenile) clustered with the majority of healthy controls and did not exhibit up-regulation of these genes.

The tight cluster of IFN-regulated transcripts that are up-regulated in most DM patients consisted of 93 genes (Figure 3). This set of transcripts is enriched for SLE IFN signature genes (43 of 93 genes). Many classical IFN-induced genes are found in this cluster, including STAT1, a transcription factor responsible for induction of IFN target genes; SOCS1, a negative regulator of the IFN pathway; the myxovirus resistance genes MX1 and MX2; and the oligoadenylate synthetase transcripts OAS1, OAS2, and OAS3. Other notable genes included the Fcγ receptor FCGR1A, the interleukin 1 receptor antagonist (IL1RN), the pro-apoptotic TNFSF10/TRAIL, and the inflammatory chemokine CXCL10/IP-10.

The DM IFN Signature is Associated with Disease Activity

Because the IFN signature is also observed in SLE and correlates with lupus activity, we first examined whether the degree of up-regulation of IFN signature genes in DM was comparable to what is observed in SLE. An IFN score was derived from the normalized expression levels of 43 transcripts that overlap between the DM and SLE IFN signatures. The IFN score was calculated for both patient groups as well as the healthy controls. Both patient groups exhibited IFN scores that were significantly higher than

Table 2. Shown are r-values from the correlation between protein levels and enzymes or disease activity. CK, creatine kinase. AST, aspartate aminotransferase. ALT, alanine aminotransferase. Stars indicate p-value derived from random permutation testing: *p<0.05, **p<0.01, ***p<0.0001.

	CK	Aldolase	AST	ALT	Activity
MCP-1	0.36	0.29	**0.69***	**0.74***	**0.55***
MCP-2	0.31	0.32	**0.70***	**0.70***	**0.60****
IP-10	0.55	**0.66***	**0.92*****	**0.73***	0.46
I-TAC	–0.10	**0.74***	0.47	0.68	0.36

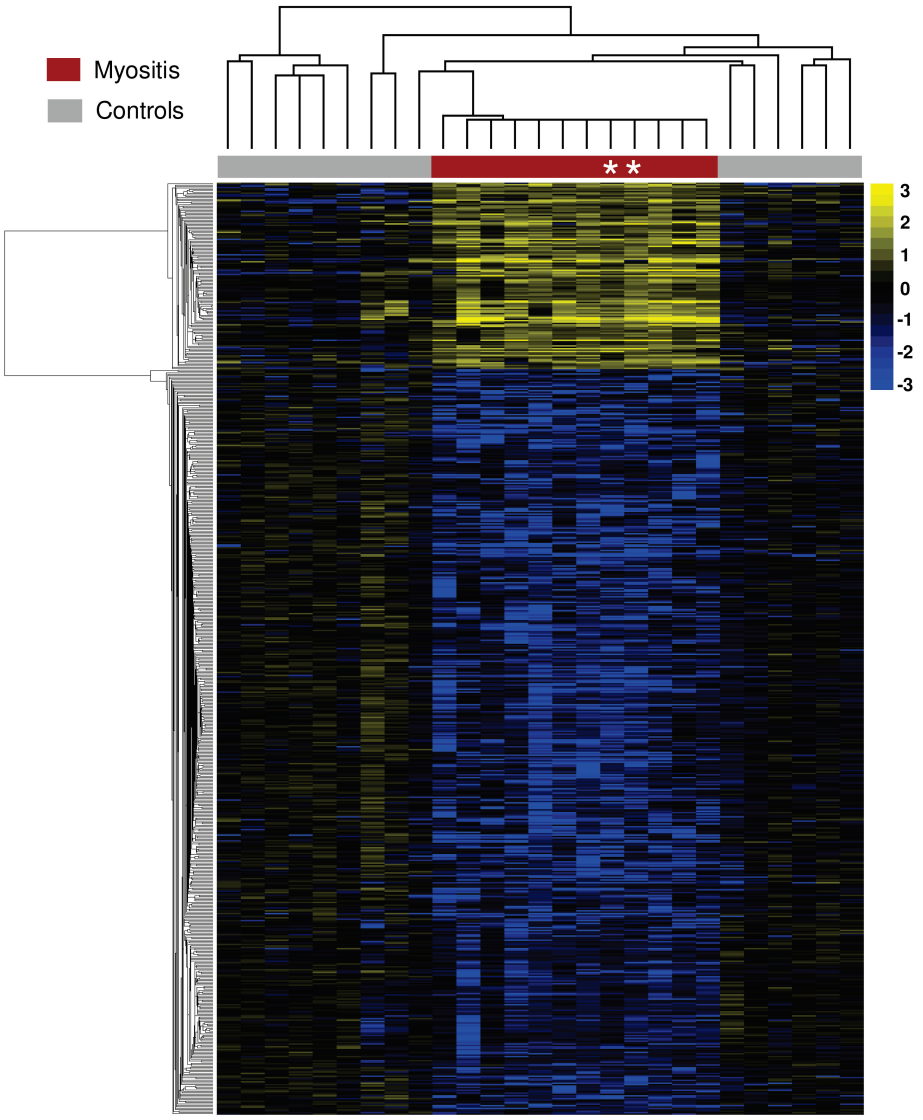

Figure 1. Gene expression patterns distinguish DM patients from normal controls. Shown are expression data from 12 patients and 15 healthy controls (in columns) for 655 genes (in rows) that were differentially expressed between DM and controls. Expression levels are represented as $\log_2$ ratios relative to the mean expression of control subjects according to the color key provided (range of linear fold change depicted is –8 to +8). Juvenile DM patients are marked by *.

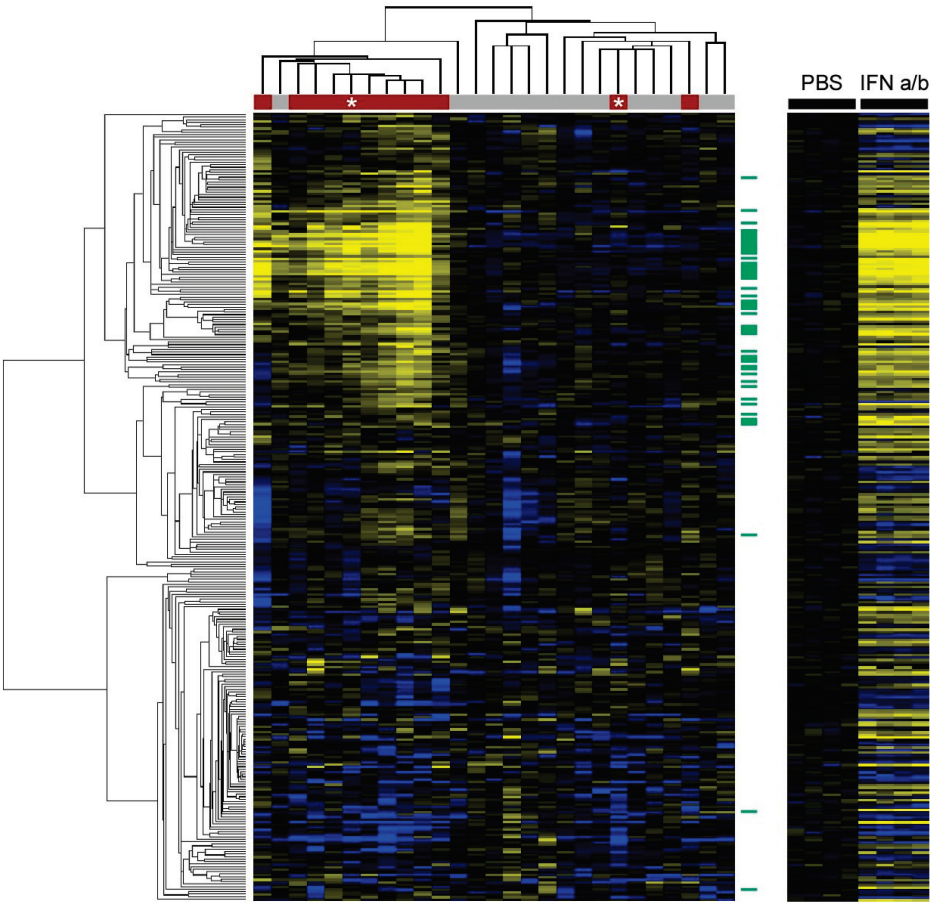

Figure 2. Elevated expression of type I IFN-regulated genes in DM. Shown in the left panel are the expression patterns of 315 IFN-regulated genes (rows) in 12 DM patients (red bars) and 15 controls (gray bars). Green bars on the right indicate genes that belong to the peripheral blood IFN signature in SLE patients. The right panel shows the expression levels of these genes in control donor PBMCs treated for 6 h in vitro with IFN-α/β or PBS control. Expression data are shown as $\log_2$ ratios relative to the mean expression of control subjects (left panel) or PBS-treated controls (right panel) according to the color key provided in Figure 1.

the control group (Figure 4; mean ± SD IFN score for controls, 5.0 ± 1.3; myositis, 14.6 ± 9.5, p-value vs. controls = 5.0×10^{-3}; SLE, 11.0 ± 6.4, p-value vs. controls = 1.1×10^{-10}). We found that the IFN signature genes were up-regulated in DM patients at a level equivalent to that seen in SLE patients (Figure 4; $P = 0.23$). When considering only the ten "IFN-positive" DM patients, the average fold-increase in expression of these 43 transcripts relative to controls was 5.3 (range 1.5- to 17-fold).

We next examined the relationship between the IFN signature and DM disease activity. For each patient, the examining rheumatologist assigned a disease activity score (0 = inactive, 1 = mild activity, 2 = high activity), based on muscle strength testing, muscle enzyme elevation, ulcerative skin disease and patient's report of functional assessment. This examiner was blinded to the IFN status of the patients. The highest IFN scores were found in those patients with the highest disease activity (Figure 5). Although the sample size is relatively small, there was a significant difference between the IFN scores of patients with high disease activity (n = 8; average IFN score = 17.2 ± 10.6) and patients with inactive disease (n = 3; average IFN score = 7.9 ± 2.7, $P = 0.05$ by unpaired t-test). Although there was a trend for positive correlation between the IFN score and disease activity (r = 0.44), the level of correlation trended toward, but did not reach statistical significance ($P = 0.06$ by random permutation of the dataset).

Individual laboratory indicators of disease were also examined for association with the IFN signature. Although it did not achieve significance, there was a trend of negative correlation between IFN score and lymphocyte count (r = -0.50, $P = 0.09$). Other trends included positive correlations between IFN score and two aminotransferases, aspartate aminotransferase (AST; r = 0.52, $P = 0.06$) and alanine aminotransferase (ALT; r = 0.77, $P = 0.008$). There was a significant positive correlation between the IFN score and oral prednisone dose (r = 0.81, $P = 0.006$), although high dose intravenous glucocorticoid therapy has been shown to ablate the IFN signature in pediatric SLE patients (13).

Serum Levels of IFN-Regulated Proteins are Associated with DM Disease Activity

We have previously identified a set of IFN-regulated proteins that are elevated in the serum of lupus patients and whose levels correlate with the IFN gene expression signature (Bauer et al., in press). We examined the levels of these proteins in the serum samples of DM patients and assessed the relationship between indicators of DM disease activity and levels of these IFN-regulated proteins. The levels of several analytes were positively correlated with the myositis IFN gene expression signature, and three analytes exhibited significant correlations with the IFN gene score (MCP-2, IP-10, and I-TAC; r > 0.58, corresponding to $P < 0.05$ by random permutation). Several IFN-regulated chemokines showed significant correlations with enzyme levels (Table 2). In particular, MCP-1/CCL2, MCP-2/CCL8, and IP-10/CXCL10 exhibited significant positive correlations with at least two en-

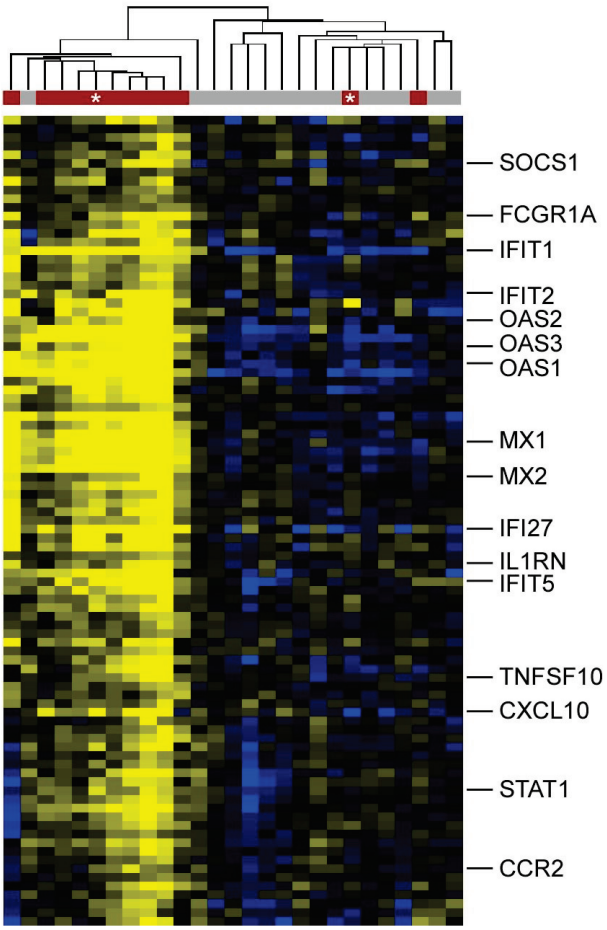

Figure 3. Shown is a magnification of a cluster of 93 IFN-regulated genes from Figure 2 that were up-regulated in DM patients and enriched for SLE type I IFN signature genes (43 of 93 genes). Selected genes are identified on the right-hand side.

zymes. In DM patients with the highest degree of disease activity, several IFN-regulated proteins were found at elevated levels (Table 2 and Figure 6). These include IP-10, which was also found to be elevated at the level of blood cell mRNA (Figure 4), MCP-1, and MCP-2.

DISCUSSION

Global gene expression profiling of peripheral blood cells has the potential to reveal clinically useful biomarkers and novel therapeutic targets for human disease. Here we have applied this technology to study transcriptional patterns in the blood of adult and juvenile DM patients, revealing several interesting functional groups of genes that are dysregulated in DM compared with healthy controls. Several immune-related transcripts previously associated with other autoimmune diseases were also implicated here in DM. The inflammatory mediator S100A8, which was increased in DM, is also found at elevated levels in patients with other autoimmune diseases, including psoriatic arthritis (29), psoriasis (30), rheumatoid arthritis (RA) (31), juvenile idiopathic arthritis (32), and type 1 diabetes (33). The B cell activating factor TNFSF13B (BAFF) is up-regulated in several autoimmune diseases, including SLE, Sjogren's syndrome, and RA (see (34) for review), and was similarly up-regulated in DM blood cells. Interestingly, several natural killer (NK) cell receptors were found at decreased levels in DM. Killer cell immunoglobulin-like receptors (KIRs) are MHC class I receptors found on natural killer cells. Combinations of HLA and KIR genes have been associated with psoriatic arthritis (35) and type 1 diabetes (36). Given that HLA genes are known to be associated with DM (2), the KIR genes may also be interesting candidates to study for potential genetic association to DM.

Several muscle-related transcripts, including myosin and related proteins, were differentially expressed in DM blood cells. Interestingly, T cells from juvenile DM patients, but not controls, are stimulated to produce TNF-α in response to epitopes from homologous sequences shared between myosin and a streptococcal protein, suggesting that myosin may be a candidate for immunomodulatory therapy (37). Another prominent group of transcripts dysregulated in DM included genes involved in mitochondrial and oxidative pathways, suggesting that disruption of oxidative balance may contribute to myositis pathogenesis. Muscle biopsy specimens from patients with DM and other inflammatory myopathies exhibit up-regulation of nitric oxide synthase on muscle fibers, which may promote oxidative stress-mediated muscle fiber damage and necrosis (38). Disrupted mitochondrial function and oxidative balance are also implicated in abnormal cell death in SLE (39).

Several important studies have used gene expression profiling to demonstrate IFN pathway activation in muscle tissue of patients with DM (5,7–9), suggesting that the IFN pathway might be a useful marker for disease and may provide new therapeutic targets. However, muscle biopsy is an expensive and invasive procedure that is not routinely performed in the clinic, especially with the evolution of magnetic resonance imaging. An alternative method for assessing the level of IFN pathway activation is desirable for more high-throughput screening and for potential use in the clinic. We demonstrate that IFN pathway activation is detectable by gene expression profiling of

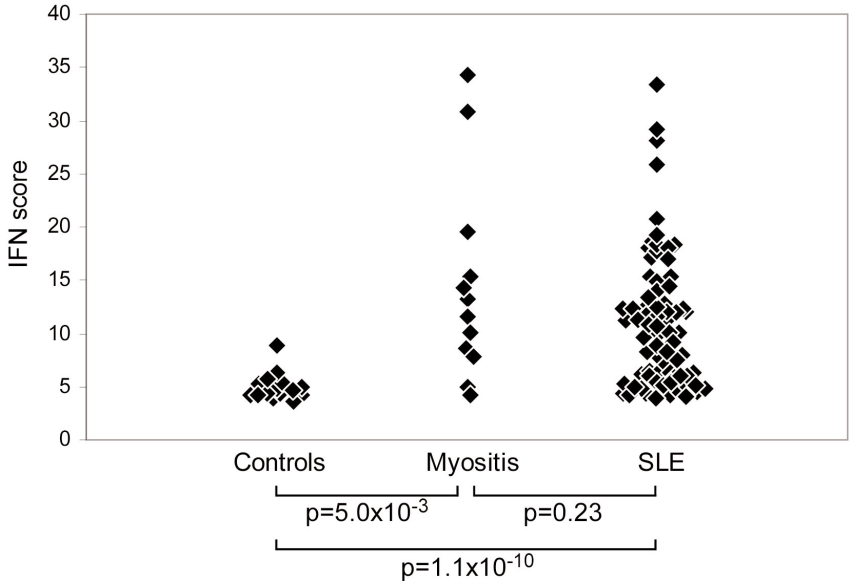

Figure 4. IFN signature genes are up-regulated in DM patients to the same degree as observed in SLE patients. Shown are IFN scores of controls (n = 15), DM patients (n = 12), and SLE patients (n = 73).

peripheral blood cells from adult and juvenile DM patients. The peripheral blood IFN signature is correlated with DM disease activity, and may be a useful tool for monitoring disease status. The levels of several IFN-regulated serum proteins also correlate with DM activity. All together, these data suggest that measurement of transcripts or serum proteins from a routine blood draw may provide a more practical and less invasive means for assessing IFN pathway activation in DM.

Interestingly, the levels of individual IFN-regulated proteins assayed in this study did not always correlate with the levels of the corresponding transcripts. We have observed a similar phenomenon in SLE patients, although the protein-derived IFN score overall remained highly correlated with the IFN gene expression score (Bauer et al., in press). The discordance between individual transcript and protein levels may reflect the short half-life of the mRNAs, or alternatively may indicate that the primary sites of protein production are extra-vascular. In the DM patients, we also noted several positive correlations between IFN-regulated chemokines and enzyme levels, but none with CK. Of the muscle enzymes, CK is the least reliable measure of disease activity, and variations occur in patients (for example, normal CK values with severe weakness and elevated aldolase).

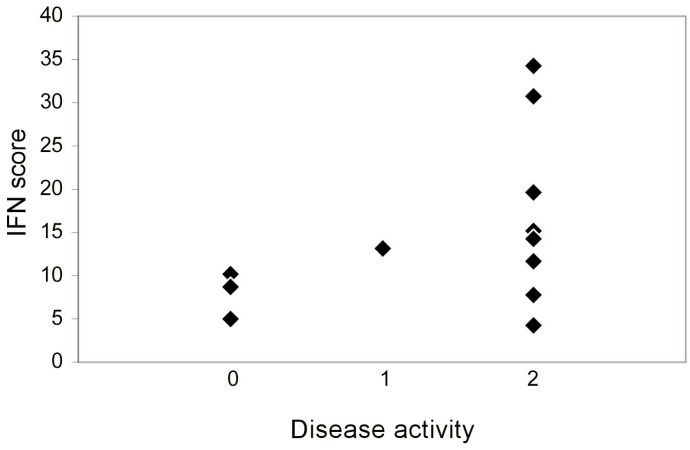

Figure 5. The IFN signature is associated with DM disease activity. Shown are IFN scores for DM patients grouped by disease activity (0 = inactive, 1 = mild activity, 2 = high activity). There were three patients in the inactive group, one patient in the mild activity group, and eight patients in the high activity group.

There are some limitations with the current study. First, the number of patients studied is relatively small, although this group of subjects is quite representative of the clinical spectrum in DM. Second, we have not been able to correlate these serum gene signatures with primary muscle tissue signatures. Biopsy material either was not available for these subjects, or was limited in quantity. Further validation of these interesting findings would be best performed in an experiment where both blood cells and muscle are examined together. Finally, we have only studied dermatomyositis in the current study, and the applicability of these findings to polymyositis, or other forms of inflammatory muscle disease (for example, inclusion body myositis) is unknown.

Type I IFN has also been implicated in disease pathogenesis of human SLE (10,11). A key aspect of the current model for IFN production in SLE is the triggering of toll-like receptors (TLR7 and 9) in PDCs. PDCs are relatively infrequent in the peripheral blood, but they are found both in the inflamed skin lesions of lupus patients (18,19) and in the affected muscle tissue of adult and pediatrics DM patients (5). PDCs produce type I IFN in response to exogenous TLR ligands, such as viral and bacterial nucleic acids or their analogs (40–42), as well as endogenous ligands (43,44). Circulating, nucleic-acid-containing immune complexes are

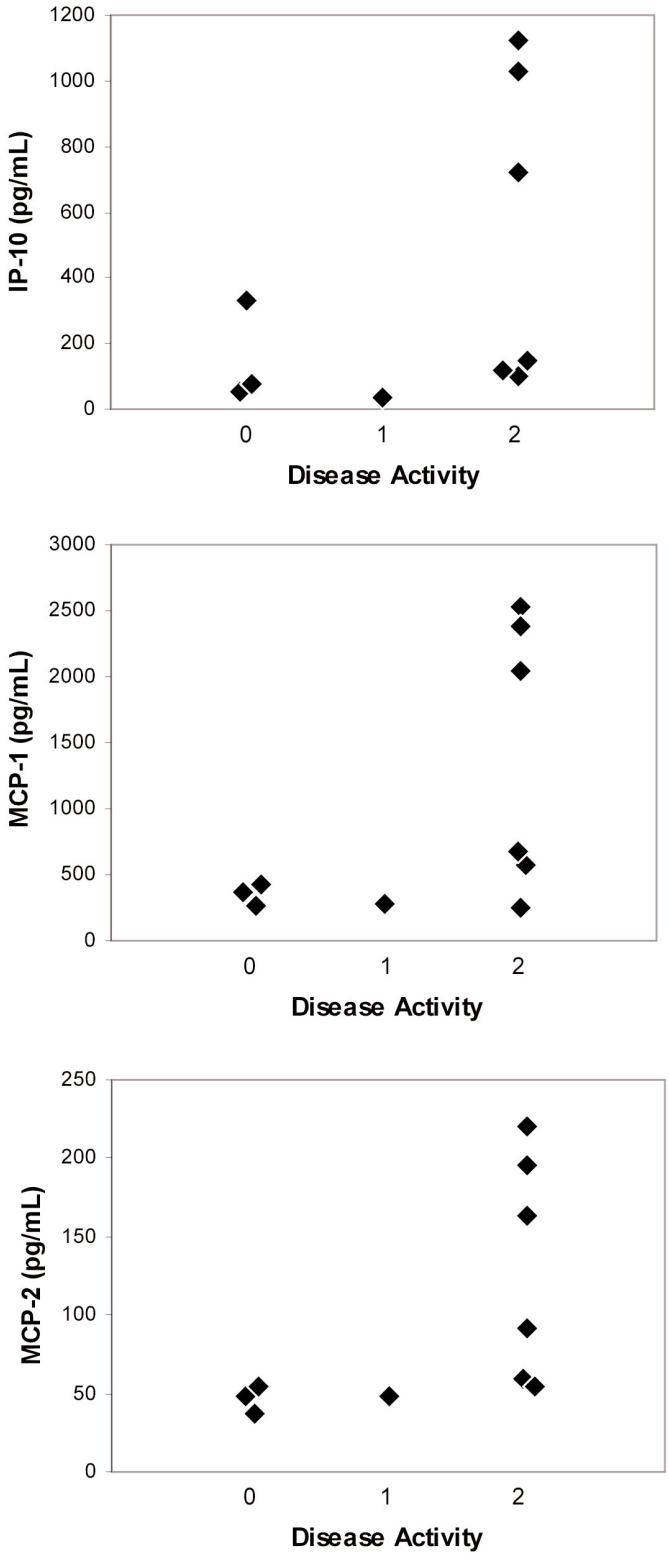

Figure 6. Levels of IFN-regulated serum proteins are associated with DM disease activity. Shown are the levels of three IFN-regulated proteins measured in the serum of 10 DM patients using Luminex xMAP technology. Patients are classified by disease activity as in Figure 5.

proposed to play a role in perpetuating the cycle of IFN production in SLE (10,11). The hypothesis that viral infections may serve as triggers for DM (4) provides a potential mechanism for the induction of type I interferon in this disease. Also, given the frequent observation of autoantibodies in DM patients, sometimes including antibodies with nuclear specificities, it is possible that nucleic-acid containing immune complexes could contribute to IFN production in these patients. Interestingly, single nucleotide polymorphisms in interferon regulatory factor 5 (IRF5), an IFN-inducible transcription factor that drives IFN induction downstream of TLR signaling, are strongly associated with SLE (45,46). The observation of the IFN signature in DM also points toward the type I IFN pathway as a candidate pathway for DM susceptibility genes.

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REFERENCES

1. Mastaglia FL, Phillips BA. (2002) Idiopathic inflammatory myopathies: epidemiology, classification, and diagnostic criteria. *Rheum. Dis. Clin. North. Am.* 28:723–41.
2. Reed AM. (2001) Myositis in children. *Curr. Opin. Rheumatol.* 13:428–33.
3. O'Hanlon TP et al. (2005) Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -DRB1 and -DQA1 allelic profiles and motifs define clinicopathologic groups in caucasians. *Medicine (Baltimore).* 84:338–49.
4. Christensen ML, Pachman LM, Schneiderman R, Patel DC, Friedman JM. (1986) Prevalence of Coxsackie B virus antibodies in patients with juvenile dermatomyositis. *Arthritis Rheum.* 29:1365–70.
5. Greenberg SA et al. (2005) Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. *Ann. Neurol.* 57:664–78.
6. Greenberg SA et al. (2002) Molecular profiles of inflammatory myopathies. *Neurology.* 59:1170–82.
7. Raju R, Dalakas MC. (2005) Gene expression profile in the muscles of patients with inflammatory myopathies: effect of therapy with IVIg and biological validation of clinically relevant genes.

Brain. 128:1887–96.
8. Tezak Z et al. (2002) Gene expression profiling in DQA1*0501+ children with untreated dermatomyositis: a novel model of pathogenesis. *J. Immunol*. 168:4154–63.
9. Zhou X, Dimachkie MM, Xiong M, Tan FK, Arnett FC. (2004) cDNA microarrays reveal distinct gene expression clusters in idiopathic inflammatory myopathies. *Med. Sci. Monit*. 10:BR191–7.
10. Baechler EC, Gregersen PK, Behrens TW. (2004) The emerging role of interferon in human systemic lupus erythematosus. *Curr. Opin. Immunol*. 16:801–7.
11. Ronnblom L, Eloranta ML, Alm GV. (2006) The type I interferon system in systemic lupus erythematosus. *Arthritis Rheum*. 54:408–20.
12. Baechler EC et al. (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. U. S. A*. 100:2610–5.
13. Bennett L et al. (2003) Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med*. 197:711–23.
14. Crow MK, Kirou KA, Wohlgemuth J. (2003) Microarray analysis of interferon-regulated genes in SLE. *Autoimmunity*. 36:481–90.
15. Dall'era MC, Cardarelli PM, Preston BT, Witte A, Davis JC Jr. (2005) Type I interferon correlates with serological and clinical manifestations of SLE. *Ann. Rheum. Dis*. 64:1692–7.
16. Kirou KA et al. (2005) Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. *Arthritis Rheum*. 52:1491–503.
17. Peterson KS et al. (2004) Characterization of heterogeneity in the molecular pathogenesis of lupus nephritis from transcriptional profiles of laser-captured glomeruli. *J. Clin. Invest*. 113:1722–33.
18. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. (2001) Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am. J. Pathol*. 159:237–43.
19. Blomberg S et al. (2001) Presence of cutaneous interferon-alpha producing cells in patients with systemic lupus erythematosus. *Lupus*. 10:484–90.
20. Baechler EC et al. (2006) Gene expression profiling in human autoimmunity. *Immunol. Rev*. 210:120–37.
21. Bohan A, Peter JB. (1975) Polymyositis and dermatomyositis (first of two parts). *N. Engl. J. Med*. 292:344–7.
22. Bohan A, Peter JB. (1975) Polymyositis and dermatomyositis (second of two parts). *N. Engl. J. Med*. 292:403–7.
23. Huber AM et al. (2004) Validation and clinical significance of the Childhood Myositis Assessment Scale for assessment of muscle function in the juvenile idiopathic inflammatory myopathies. *Arthritis Rheum*. 50:1595–603.
24. Oddis CV et al. (2005) International consensus guidelines for trials of therapies in the idiopathic inflammatory myopathies. *Arthritis Rheum*. 52:2607–15.
25. Petri M, Genovese M, Engle E, Hochberg M. (1991) Definition, incidence, and clinical description of flare in systemic lupus erythematosus. A prospective cohort study. *Arthritis. Rheum*. 34:937–44.
26. Hochberg Y, Benjamini Y. (1990) More powerful procedures for multiple significance testing. *Stat. Med*. 9:811–8.
27. Tusher V, Tibshirani R, Chu G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl. Acad. Sci. U. S. A*. 98:5116–21.
28. Eisen MB, Spellman PT, Brown PO, Botstein D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. U. S. A*. 95:14863–8.
29. Batliwalla FM et al. (2005) Microarray analyses of peripheral blood cells identifies unique gene expression signature in psoriatic arthritis. *Mol. Med*. 11:21–9.
30. Semprini S et al. (2002) Evidence for differential S100 gene over-expression in psoriatic patients from genetically heterogeneous pedigrees. *Hum. Genet*. 111:310–3.
31. Batliwalla FM et al. (2005) Peripheral blood gene expression profiling in rheumatoid arthritis. *Genes Immun*. 6:388–97.
32. Schulze zur Wiesch A et al. (2004) Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. *Clin. Exp. Rheumatol*. 22:368–73.
33. Bouma G, Lam-Tse WK, Wierenga-Wolf AF, Drexhage HA, Versnel MA. (2004) Increased serum levels of MRP-8/14 in type 1 diabetes induce an increased expression of CD11b and an enhanced adhesion of circulating monocytes to fibronectin. *Diabetes*. 53:1979–86.
34. Kalled SL. (2005) The role of BAFF in immune function and implications for autoimmunity. *Immunol. Rev*. 204:43–54.
35. Nelson GW et al. (2004) Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. *J. Immunol*. 173:4273–6.
36. van der Slik AR et al. (2003) KIR in type 1 diabetes: disparate distribution of activating and inhibitory natural killer cell receptors in patients versus HLA-matched control subjects. *Diabetes*. 52:2639–42.
37. Massa M et al. (2002) Self epitopes shared between human skeletal myosin and Streptococcus pyogenes M5 protein are targets of immune responses in active juvenile dermatomyositis. *Arthritis Rheum*. 46:3015–25.
38. Tews DS, Goebel HH. (1998) Cell death and oxidative damage in inflammatory myopathies. *Clin. Immunol. Immunopathol*. 87:240–7.
39. Perl A, Gergely P Jr., Nagy G, Koncz A, Banki K. (2004) Mitochondrial hyperpolarization: a checkpoint of T-cell life, death and autoimmunity. *Trends Immunol*. 25: 360–7.
40. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science*. 303:1529–31.
41. Heil F et al. (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science*. 303:1526–9.
42. Hemmi H et al. (2000) A Toll-like receptor recognizes bacterial DNA. *Nature*. 408:740–5.
43. Bave U, Alm GV, Ronnblom L. (2000) The combination of apoptotic U937 cells and lupus IgG is a potent IFN-alpha inducer. *J. Immunol*. 165:3519–26.
44. Lovgren T, Eloranta ML, Bave U, Alm GV, Ronnblom L. (2004) Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis. Rheum*. 50:1861–72.
45. Graham R et al. (2006) A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. *Nat. Genet*. 38:550–5.
46. Sigurdsson S et al. (2005) Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *Am. J. Hum. Genet*. 76:528–37.