Gene Expression Profiling in Blood and Affected Muscle Tissues Reveals Differential Activation Pathways in Patients with New-onset Juvenile and Adult Dermatomyositis

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ABSTRACT. ***Objective.*** To identify shared and differential molecular pathways in blood and affected muscle between adult dermatomyositis (DM) and juvenile DM, and their association with clinical disease activity measures.

Methods. Gene expression of transcription factors and cytokines involved in differentiation and effector function of T cell subsets, regulatory T cells and follicular Th cells, were analyzed in the blood from 21 newly diagnosed adult and 26 juvenile DM subjects and in 15 muscle specimens (7 adult and 8 juvenile DM) using a custom RT2 Profiler PCR Array. Disease activity was determined and measured by established disease activity tools.

Results. The most prominent finding was the higher blood expression of Th17-related cytokines [retinoic acid-related orphan receptor-γ, interferon regulatory factor 4, interleukin (IL)-23A, IL-6, IL-17F, and IL-21] in juvenile DM at baseline. In contrast, adult patients with DM showed increased blood levels of STAT3 and BCL6 compared with juvenile DM. In muscle, GATA3, IL-13, and STAT5B were found at higher levels in juvenile patients with DM compared with adult DM. Among 25 patients (11 adult and 14 juvenile DM) who had blood samples at baseline and at 6 months, increased expression of *IL-1β, STAT3, STAT6, STAT5B*, and *BCL6* was associated with an improvement in global extramuscular disease activity.

Conclusion. We observed differences in gene expression profiling in blood and muscle between new-onset adult and juvenile DM. Cytokine expression in the blood of juvenile patients with new-onset DM was dominated by Th17-related cytokines compared with adult patients with DM. This may reflect the activation of different Th pathways between muscle and blood. (First Release November 1 2016; J Rheumatol 2017;44:117–24; doi:10.3899/jrheum.160293)

Key Indexing Terms:
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Dermatomyositis (DM) is a systemic, inflammatory disorder primarily affecting muscle and skin in children and adults. Although the pathogenesis of adult and juvenile DM appear similar, there are important differences in the clinical features and pathophysiology. Juvenile DM has more cutaneous features such as nailfold telangiectasia and calcinosis with a

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good outcome in the majority of the cases[1], whereas adult DM is more resistant to treatment and complete remission is infrequent with a higher mortality rate[2,3,4]. Similarly, adult patients with DM have a higher risk of developing cancer, but malignancies are rarely reported in juvenile DM[5]. Interstitial lung disease is a common occurrence in adult DM in up to 23.8% of cases[6], but it is uncommon in juvenile DM.

The cellular infiltrates are reported to be similar in adult and juvenile DM and consist of CD4+ T cells, plasmacytoid dendritic cells, B cells, macrophages, and Th17 cells.

Naive CD4+ T cells develop into functionally mature effector cells upon stimulation with relevant antigenic peptides that induce differentiation into at least 4 major Th lineages including Th1, Th2, Th17, and Foxp3+ Treg cells, which produce lineage-indicating cytokines and perform distinct functions in regulating immunity and inflammation[7,8,9]. In addition, other Th cell subsets have been recently described, such as follicular Th (TFH) cells, Th9 cells, and interleukin (IL) 22-expressing Th22 cells[10,11,12].

The main way that differentiated CD4 T cells regulate inflammation is by the release of cytokines. Cytokines and transcription factors are critical for determining CD4+ T cell fates and the effector cytokine production. For instance, IL-12 induces expression of the transcription factors T-bet (TBX21) and signal transducer activator of transcription 4 (STAT4), which mediates the differentiation of Th1 cells with production of interferon-γ (IFN-γ), IL-2, and tumor necrosis factor-α (TNF-α) and -β, whereas IL-4 induces the transcription factors GATA-binding protein 3 (GATA3) and STAT6 with subsequent differentiation of Th2 cells and production of cytokines IL-4, IL-5, IL-13, and IL-10[13,14]. Th17 cells express the master transcription regulator retinoic acid-related orphan receptor-γ (RORC) and produce canonical IL-17A and IL-17F cytokines. The cytokines IL-6, IL-21, IL-23, and transforming growth factor-β1 (TGF-β1) are crucial factors for the differentiation of Th17 cells[8,15]. Cytokine production of these Th subsets can be heterogeneous and overlapping, with individual cells within a given polarized Th population at any 1 timepoint not necessarily secreting the full range of cytokine for that subset[16].

Studies concerning cytokine expression in muscle tissue of patients with inflammatory myopathies including DM have shown the dominant presence of IL-1α, TGF-β1, IL-6, and proinflammatory Th1 cytokines (IFN-γ, IL-2), and a weak expression of Th2-derived cytokines[17,18,19,20], suggesting a Th1/Th2 balance in muscle tissue with a greater Th1 response in DM. Interestingly, a study showed that CD4+IFN-γ+ cells were decreased whereas the CD4+IL-4+ cells (Th2 cells) were increased in the blood of patients with new-onset DM[21], suggesting that Th2 cells may predominate in the blood of active DM. In parallel to Th1 and Th2 cells, studies have begun to elucidate the importance of IL-17–producing Th17 cells in the pathogenesis of myositis[18,22,23].

Although it appears that the pathogenesis of DM in adults and children is similar, striking differences in clinical features, outcome, and response to treatment exist and suggest that different mechanisms may be at least partially involved. Only a few studies have previously compared the immunopathological features of adult and juvenile DM; most studies combine these diseases. In our study, we sought to compare mRNA expression differences between adults and children with new-onset DM in genes related to Th cell signaling pathways and/or innate immune response using gene expression profiles obtained from peripheral blood mononuclear cells (PBMC) and affected muscle tissues. We also sought to determine whether changes existed in gene expression profiles between baseline visit and 6 months of followup in both DM groups and how they related to changes in clinical disease activity. The purpose of our study was to extend our previous findings[24] to include both gene expression data obtained from blood and affected muscle samples to assess which "pathways" or individual genes were deregulated in juvenile and adult DM.

MATERIALS AND METHODS

Blood samples and clinical data were obtained prospectively from 26 children and 21 adults with new-onset disease (< 6 mos of clinical symptoms) who fulfilled the Bohan and Peter criteria[25] for the diagnosis of DM at baseline. Blood samples were available in 25 patients (11 adults and 14 juveniles) at baseline and 6 months. Of the 47 patients, 8 were included in a previous report[24] and 39 were new samples. All subjects had a definitive diagnosis of DM and were seen at the Division of Rheumatology at the Mayo Clinic, Rochester, Minnesota. The Mayo Clinic Institutional Review Board approved this study (10-005501), and informed consent was obtained from each participant. Disease activity measures included the extramuscular disease activity, physician's global activity (PGA), and the manual muscle testing of 8 muscle groups[24]. PBMC were collected at baseline and at a 6-month followup visit. In addition, paired muscle biopsies were obtained from 7 adults and 8 juvenile patients with DM at baseline visit. Biopsies were obtained from the vastus lateralis in 7 patients (all juvenile DM) and from the deltoid and triceps muscles for 8 (7 adult DM and 1 juvenile DM). These specimens were reviewed and confirmed to be DM by a neuromuscular pathologist (neuromuscular laboratory, Mayo Clinic, Rochester, Minnesota, USA).

Gene expression profiling. Whole blood was collected in tubes treated with an RNA stabilization agent (PAXgene; PreAnalytiX). 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.). Muscle was homogenized using a PowerGen 700 (Fisher Scientific) and RNA isolated by an organic extraction method (TRIzol; Life Technologies), and was further purified with RNeasy Mini Kit (Qiagen) and an on-column DNase treatment. We measured mRNA expression of *IL-1β*, *IL-6*, *IL-23A*, *IL-27*, *IL-17A*, *IL-17D*, *IL-17F*, *IL-21*, *IL-22*, *RORC*, *TGF-β1*, *IFN-γ*, *TNF-α*, *IL-2*, *TBX21*, *STAT4*, *IL-4*, *IL-5*, *IL-10*, *IL-12B*, *IL-13*, *STAT6*, *GATA3*, interferon regulatory factor 4 (*IRF4*), forkhead box P3 (*FOXP3*), *STAT5B*, *IL-10*, *IL-9*, B cell CLL/lymphoma 6 (*BCL6*), *STAT3*, lymphotoxin-α (*LTA*/*TNFSF1*), and the housekeeping genes *GAPDH*, *B2M*, and *ACTB* using a custom RT2 Profiler PCR Array and amplified on an ABI 7900HT PCR system. The genes we analyzed were grouped in "pathways" or functionally related groups of genes according to the particular cytokines secreted by the individual Th cell subset as well as innate immune cells (Table 1). The transcription factors examined were unique to specific Th cell pathways such as *TBX21* (Th1), *GATA3* (Th2), RORC (Th17), and *FOXP3* (Treg). However, we are aware that most genes can act as part of 1 or more pathways. For instance, *IRF4* expression, a transcription factor

Table 1. Functional grouping of genes analyzed in blood and muscle tissue of adult and juvenile patients with dermatomyositis.

Signaling Pathway	Master Transcriptional Factor	Genes
Th1 subset-related cytokines	*TBX21*	*IFN-γ*, *TNF-α*, *TNFSF1/LTA*, *IL-2B*, *IL-12B*, *STAT4*
Th2 subset-related cytokines	*GATA3*	*IL-4*, *IL-5*, *IL-10*, *IL-13*, *IRF4*, *STAT6*
Th17 subset-related cytokines	*RORC*	*IL-23A*, *IL-17A*, *IL-17D*, *IL-17F*, *IL-1B*, *IL-6*, *TGF-β1*, *IL-9*, *IL-22*, *IL-21*, *IL-27*, *IL-10*, *IRF4*, *STAT3*
Transcriptional factors for Treg cells	*FOXP3*	*IL-10*, *TGF-β1*, *STAT5B*
Transcriptional regulator for TFH cells	*BCL6*	*IL-21*, *STAT3*, *IRF4*

TFH: follicular Th.

essential for Th2 effector cell differentiation, is also known to interact with *STAT3* to induce *RORγt* expression, suggesting its effect on Th17 cells[26].

Statistical analysis. Data are expressed as the median (range) where indicated. A Wilcoxon rank sum test was performed for comparisons between 2 groups (e.g., adult vs juvenile DM). Paired Student t tests were performed to compare gene expression levels between baseline and 6 months as well as between blood and muscle samples. Spearman correlation coefficients were used to examine correlations between variables. P values < 0.05 were considered statistically significant. Multiple comparisons involved a maximum of 30 comparisons for any specific hypothesis; thus, the Bonferroni correction would consider p values < 0.0017 to be significant. Analyses were performed using SAS version 9.3 (SAS Institute Inc.) and R 3.1.1 (R Foundation for Statistical Computing).

RESULTS

Demographic and clinical characteristics of the patients with DM. Demographic and clinical characteristics of the patients with DM are summarized in Table 2. At baseline, patients in both groups had similar disease activity as evidenced by the values for the disease activity core measures. Ten adult patients with DM (48%) and 6 juvenile patients with DM (29%) had begun treatment with 1 or more traditional disease-modifying antirheumatic drugs (DMARD) in the 2 months prior to the baseline visit. Concomitant corticosteroid use was reported for 14 adults (70%) and 8 children (38%) with DM.

Overall, these patients improved substantially on all disease activity measures between baseline and the 6-month followup visit, with a median improvement of 20/100 in the extramuscular visual analog scale (VAS), 22/100 in the muscle VAS, and 34/100 in the global VAS. Changes in disease activity measures were not significantly different between adults and children with DM (p = 0.14, p = 0.51, and p = 0.07 for PGA, muscle activity, and extramuscular disease activity VAS scores, respectively).

T cell lineage gene expression in blood of new-onset adult and juvenile DM. We investigated whether the T cell lineage gene expression profiling in blood would reveal distinct T cell pathways in juvenile versus adult DM. Gene expression levels of *RORC/RORγt*, the master regulator for Th17 cells, and *IRF4*, another transcription factor required for Th17 cell differentiation, were significantly increased in blood of juvenile compared with adult patients with DM at baseline (p = 0.001 and p < 0.001, respectively; Figure 1). In addition, juvenile DM had significantly higher levels of *IL-23A* (p < 0.001), *IL-6* (p < 0.001), *IL-17F* (p = 0.005), and *IL-21* (p = 0.012) mRNA at the baseline visit when compared with adult patients with DM. Similarly, *FOXP3* and *STAT4* gene levels were significantly increased in the blood of juvenile DM compared with adult DM (p = 0.016 and p = 0.009, respectively). In adult DM blood, increased levels of *STAT3* and *BCL6* were observed compared with juvenile DM blood (p = 0.028 and p = 0.002, respectively; Figure 1).

Table 2. Characteristics at baseline of adult and juvenile patients with DM. Values are mean ± SD unless otherwise specified.

Variable	Adult DM, n = 21	Juvenile DM, n = 26	p
Age at baseline, yrs	50.2 ± 17.8	9.7 ± 4.4	< 0.001
Female, n (%)	17 (81)	16 (62)	0.15
Disease duration, days, median (IQR)	61 (6, 303)	17 (0, 818)	0.86
White, n (%)	20 (95)	20 (77)	0.08
Corticosteroid use, n/n available (%)	14/20 (70)	8/21 (38)	0.04
Immunosuppressive agent use, n/n available (%)	10/21 (48)	6/21 (29)	0.20
Myositis autoantibody, n positive/n tested (%)			
Antinuclear antibodies	10/20 (50)	12/18 (67)	0.30
Anti-Jo1 antibodies	1/21 (5)	1/21 (5)	1.0
Anti-RNP antibodies	1/19 (5)	1/18 (6)	0.97
Anti-dsDNA antibodies	1/15 (7)	0/16 (0)	0.29
Anti-SSA antibodies	4/20 (20)	1/18 (6)	0.19
Anti-SSB antibodies	1/20 (5)	0/18 (0)	0.34
Global extramuscular disease activity, 0–100	31.1 ± 20.6	31.1 ± 20.5	0.95
Muscle disease activity, 0–100	34.0 ± 27.2	28.6 ± 29.2	0.41
Physician's global disease activity, 0–100	37.5 ± 23.5	36.5 ± 36.9	0.76

DM: dermatomyositis; IQR: interquartile range.

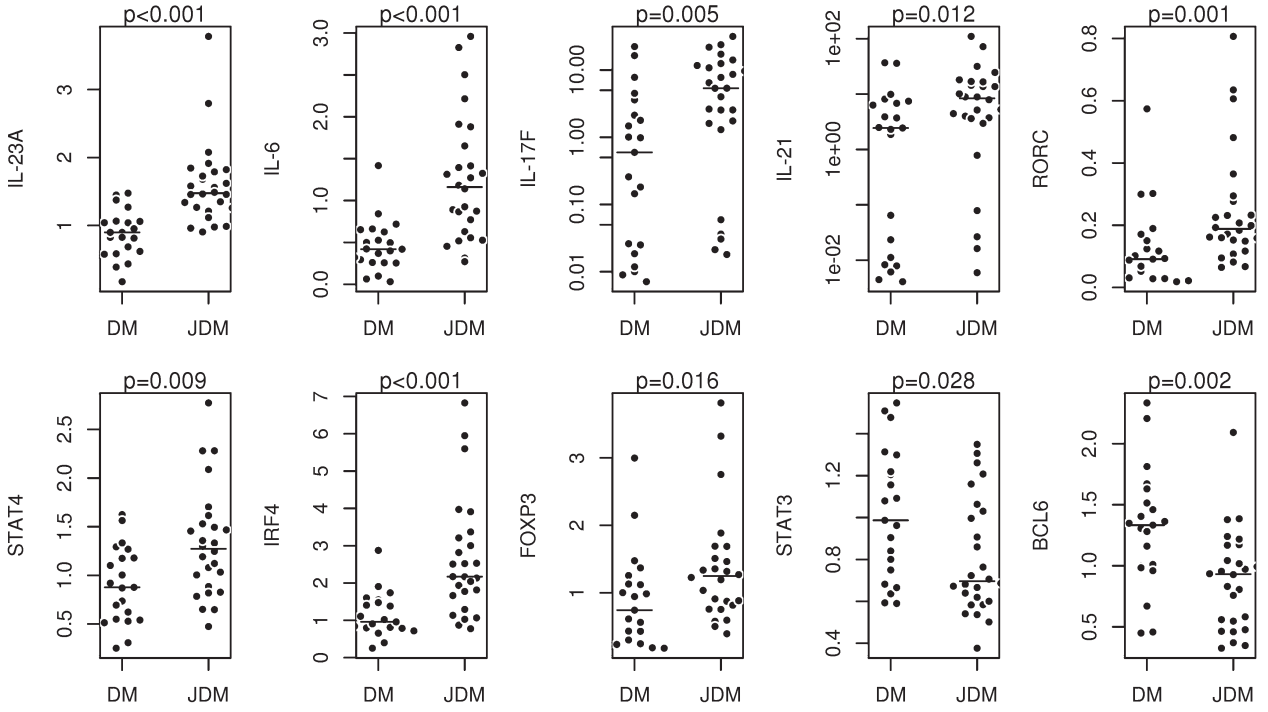

Figure 1. Gene expression profile in peripheral blood mononuclear cells of adult and juvenile patients with DM at baseline. The plot depicts the gene expression data from genes that were differentially expressed in blood between juvenile and adult DM. P values < 0.05 were considered statistically significant. Genes expressed as relative quantification. DM: dermatomyositis; JDM: juvenile DM.

T cell lineage gene expression in affected muscle of patients with new-onset DM. As a step toward understanding the differences between juvenile and adult DM, we analyzed gene expression in paired muscle biopsies collected from 15 patients with DM (7 adults and 8 children) at baseline. All DM samples were analyzed histologically to confirm DM. Perivascular lymphocytic infiltration was seen in all DM samples. A gene expression profile was also evaluated in the skeletal muscle tissues from otherwise healthy patients undergoing an orthopedic procedure. The majority of transcripts were found at higher levels in affected muscle tissue of both adult and juvenile DM compared with normal muscle tissues (data not shown).

Evidence of Th2 T cells in muscle tissue was demonstrated by increased expression levels of *GATA3* (p = 0.004) and *IL-13* (p = 0.049), and Treg cells by increased *STAT5B* expression (p = 0.049) in affected muscle of juvenile compared with adult patients with DM.

Correlation of T cell lineage gene expression with medication use in patients with DM. Glucocorticoids are critical regulators of several inflammatory cytokines[27,28], but our previous data on cytokine protein levels suggest that glucocorticoid treatment does not statistically alter the levels except for *IL-1β*[24]. Herein, we analyzed the association between glucocorticoid treatment and the expression of transcription factors. *GATA3, IRF4*, and *FOXP3* levels were lower in juvenile DM blood from patients who were receiving glucocorticoids versus those not taking glucocorticoids (p = 0.015, p = 0.001, and p < 0.001, respectively). If we looked at just patients (both juvenile and adult DM) who were taking glucocorticoids, *IL-23A*, *IL-6*, *RORC*, *IRF4*, and *BCL6* gene expression levels remained significant when comparing juvenile and adult DM; however, other comparisons that no longer reached statistical significance were marginal: *IL-17F* (p = 0.056), *IL-21* (p = 0.12), *STAT4* (p = 0.088), and *FOXP3* (p = 0.056), which could be related to the limited sample size. Only *STAT3* was clearly no longer significant (p = 0.45).

Correlation of blood gene expression profiles with clinical features in juvenile and adult DM at baseline and at 6-month followup visit. To examine whether differential gene expression was associated with DM disease activity, we compared expression with disease activity at baseline, and examined whether changes in gene expression correlated with changes in the disease activity over time. At baseline in the adults, the gene expression levels of these were found to correlate significantly with muscle disease activity measured at baseline (Figure 2): *IL-23A* (p = 0.033), *RORC* (p < 0.001), *TGF-β1* (p = 0.035), *IL-27* (p = 0.003), *IL-22* (p = 0.006), *GATA3* (p = 0.045), *IRF4* (p < 0.001), *IL-4* (p < 0.001), *IL-13* (p < 0.001), *IFN-γ* (p = 0.003), *TNF-α* (p < 0.001), *STAT4* (p = 0.006), and *FOXP3* (p = 0.032). Among adults, levels of *RORC* (p < 0.001), *IFN-γ* (p = 0.008), *STAT4* (p = 0.011), *IL-4* (p = 0.009), and *IL-13* (p < 0.001) were found to

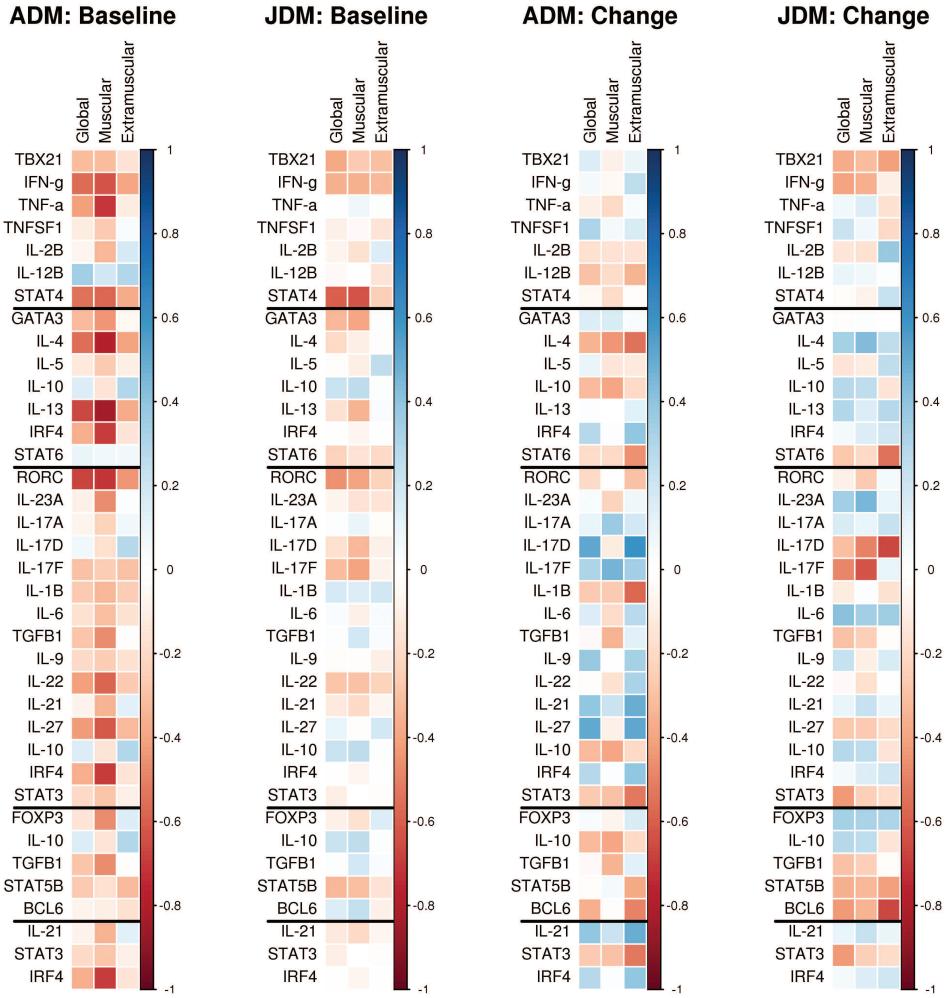

Figure 2. Correlation of mRNA expressions of T cell lineage transcription factors against measures of disease activity (global, muscular, and extramuscular) cytokines in whole blood of juvenile and adult patients with DM. The 2 leftmost panels correlate baseline gene expression values with baseline disease activity measures. The 2 rightmost panels correlate changes in gene expression from baseline to the 6-month visit with changes in disease activity measures over the same period. Negative correlations between changes in gene expression and changes in disease activity indicate that improvements in disease activity were associated with increases in gene expression levels. ADM: adult dermatomyositis; JDM: juvenile dermatomyositis.

correlate significantly with PGA at baseline and only *RORC* ($p = 0.045$) correlated significantly with extramuscular disease activity. In contrast, at baseline in the juvenile DM group, gene expression levels of *RORC* ($p = 0.042$), *IL-17F* ($p = 0.040$), *GATA3* ($p = 0.044$), and *STAT4* ($p < 0.001$) were found to correlate significantly with muscle disease activity measured at baseline (Figure 2). Only *RORC* ($p = 0.018$) and *STAT4* ($p = 0.001$) correlated significantly with global disease activity among the juvenile DM and no significant correlations with extramuscular disease were found.

Followup data were available in a total of 25 patients (11 adult and 14 juvenile DM). Among the adults, changes in *IL-17D* ($p = 0.048$) between baseline and the 6-month visit were positively correlated with changes in global extramuscular disease activity, indicating that improved disease activity was associated with decreases in gene expression levels (Figure 2). Among the juvenile patients with DM, changes in *STAT6* ($p = 0.044$), *IL17-D* ($p = 0.010$), and *BCL6* ($p = 0.009$) correlated negatively with changes in global extramuscular disease activity. No significant correlations between changes in gene expression levels and changes in muscle or PGA measures were found for either group.

Associations between changes in treatment from baseline to 6 months and cytokine gene expression levels were examined longitudinally in the adult and juvenile DM groups combined to enhance statistical power. The addition of DMARD ($n = 22$) was associated with increased levels of *IL-1β* ($p = 0.012$), *STAT3* ($p = 0.005$), *STAT6* ($p = 0.001$), and *STAT5B* ($p = 0.037$), and decreased levels of *IFN-γ* ($p = 0.027$), *IL-22* ($p = 0.044$), and *IRF4* ($p = 0.023$). Use of

glucocorticoids (n = 21) was associated with increases in *FOXP3* (p = 0.020), *IL-23A* (p = 0.013), *IRF4* (p = 0.013), and *TGF-β1* (p = 0.022), and decreases in *IL-2* (p = 0.017). Comparisons of cytokines and transcription factors between blood and muscle did now show significant differences (data not shown).

DISCUSSION

Classically, immunohistochemical and molecular (i.e., real-time PCR) analyses in DM muscle show predominant expression of macrophage-derived cytokines (i.e., IL-1, TNFSF1, and TNF-α), as well as cytokines that can originate from either T cells or macrophages (i.e., IL-6 and TGF-β1)[29,30,31]. Further, current evidence increasingly supports alternative pathogenic mechanisms, including both adaptive and innate immune-mediated responses. An innate immune response characterized by infiltration of plasmacytoid dendritic cells in DM muscle lesions and IFN-α/β–inducible gene signature in whole blood and muscle may be an important part of the pathogenesis of DM[17,32,33,34,35,36].

At present, PCR-based arrays allow facile analysis of expression of a relatively large number of pathway- or disease-focused genes with remarkable sensitivity. Here, we analyzed the expression profile of 30 genes in whole blood from 47 individuals with newly diagnosed DM and in 15 paired muscle samples. To our knowledge, our study constitutes the most comprehensive analysis contrasting the gene expression signatures between adults and children with DM to date.

Cellular and protein effectors of innate and adaptive immunity are found in the blood and inflamed muscle lesions of patients with DM, and an increasing body of evidence indicates that they are directly involved in myofiber injury[32,37]. We have previously identified a strong correlation between IL-6 serum levels and Type I IFN signature in adult and juvenile DM[23]. Similarly, a strong expression of IL-1α, IL-1β, TGF-β, and TNF-α has been observed in the affected muscle of patients with DM and other types of myositis, suggesting that these cytokines are important contributors to the pathogenesis of DM[19,38,39,40]. On the side of the adaptive immune system, despite that muscle-infiltrating T cells are predominantly CD4+ T cells in both adults and children with DM, Th1-derived cytokine IFN-γ and Th2-derived cytokine IL-4 are less abundantly expressed compared with other cytokines[30,41], suggesting that other effector T cell lineages might be involved in the pathogenesis or the possible perpetuation of the inflammatory response in DM. Although juvenile and adult DM have a set of common genetic risk alleles and abnormalities in both B and T cell functions, they also have important differences in clinical features and associated disorders, suggesting that specific immunological aberrations may differ for each disease. Our combined analysis of gene expression data in peripheral blood and muscle tissues obtained simultaneously provides a deeper understanding of the T cell pathways that regulate the pathogenesis of both myositis subsets, a finding that eventually could lead to new strategies to facilitate improvement of the therapy.

Herein, we observed differences in gene expression profiling in the blood between juvenile and adult patients with DM. The gene expression profiling that we found in the blood of juvenile patients with DM indicates a Th17 type of inflammatory process. The proportion of patients using glucocorticoids at baseline was higher in adult DM. Despite this difference, after adjusting for glucocorticoid use, we found that *IL-23A, IL-6, RORC,* and *IRF4* gene expression levels remained significantly different in juvenile DM compared with adult DM. The upregulation of Th2-related genes was apparent in the affected muscle of juvenile patients with DM compared with adult DM. However, the interpretation of gene expression profiling in muscle biopsies can be affected by several factors such as a small amount of muscle tissue or the variability of biopsy sampling of inflammatory cells.

Our study also examined the effect of DMARD and glucocorticoid use on the cytokine levels from baseline to 6-month followup. Use of DMARD was associated with increases in blood levels of *IL-1B, STAT3, STAT6*, and *STAT5B*, and with decreases in blood levels of *IFN-γ*, *IL-22*, and *IRF4*. Use of glucocorticoids was associated with increases in blood levels of *FOXP3, IL-23A, IRF4*, and *TGF-β1*, and with decreases in *IL-2*, suggesting that various potential immune pathways are altered with treatment.

In addition to the cytokines they produce, effector T cells can be distinguished by their differential expression of specific transcription factors and STAT proteins, which induce gene expression of distinct inflammatory pathways. However, although 1 transcription factor might be the predominant initiator of a differentiation pathway, effector T cells might respond to additional cytokines such as IL-2, IL-6, and IL-21 within the inflammatory environment, which might activate additional T cell lineages. For instance, FOXP3+ Tregs can produce IL-17 when activated in the presence of IL-1β and IL-6, losing their suppressive activity and promoting inflammation[42]. Additionally, there is much more T cell plasticity than previously recognized wherein a transcription factor may be involved in more than 1 particular differentiation pathway. For example, a study showed that TBX21 (Th1 lineage) and RORC (Th17 lineage) transcription factors were found to be expressed in Th17- and Th1-like Treg cells, respectively[43].

In our study, in addition to increased expression of Th17 cytokine genes, *RORC* and *IRF4* (both with critical involvement in Th17 development) were increased in juvenile DM blood compared with adult DM blood. *FOXP3* (Tregs) and *STAT4* (Th1) gene levels were also elevated in juvenile DM blood.

In contrast, we found higher expression of *BCL6* in adult DM blood compared with juvenile DM, which remained

significant after adjusting for glucocorticoid use. We observed that *STAT3* expression was higher in adult DM blood compared with juvenile DM, although not significant after adjusting for glucocorticoid use. *STAT3* is a critical factor for not only activating the proinflammatory Th17 lineage, but for promoting *BCL6* transcription and TFH differentiation[44]. TFH cells are required for germinal center formation, and studies have indicated that TFH is critical in the pathogenic autoantibody production of several autoimmune diseases[45,46]. *BCL6* is upregulated in TFH cells and is considered a master regulator for this lineage. TFH cells have been documented in peripheral blood of juvenile and adult patients with DM[47,48]. Additionally, we have previously shown that CD4+ T cells and activated B cells come together in ectopic lymphoid aggregates in affected muscle tissue of juvenile patients with DM[34], and others have documented similar findings in adult DM[49]. Together, it seems likely that circulating TFH cells could migrate and local TFH cell-B cell interactions in inflamed muscle tissue may contribute to extranodal immune activation.

In addition to its established role as a transcription factor in TFH differentiation, IL-6–Janus kinase and signal transducer and activator of transcription 3 (JAK-STAT3) activation in muscle cells is strongly associated with skeletal muscle wasting in cancer and other conditions associated with high IL-6 levels[50]. Cancer and muscle wasting are common features in adult patients with DM; however, whether the IL-6–JAK-STAT3 pathway is a primary mediator of these clinical manifestations in adults with DM needs further investigation.

The differences in gene expression profiling found between juvenile and adult DM can subsequently be applied to gain a better understanding of the complex cellular components involved in the pathogenesis. This can be applied to further studies using emerging technologies such as single cell analysis of circulating immune cell subset and/or molecular analysis of selected cell aggregates in affected muscle tissue using laser identification microdissection. Those analyses could clarify which pathways are shared between juvenile and adult DM, and which differ, identifying particular immune pathways that are active in subsets of patients with DM leading to individualized patient treatment.

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