Serum cytokine profiles of adults with idiopathic inflammatory myopathies

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Abstract

Objective

There is a paucity of available biomarkers of disease activity in idiopathic inflammatory myopathies (IIM), and serum cytokines/chemokines hold potential as candidate biomarkers. We aimed to determine serum cytokine profiles of IIM patients with active disease as compared to patients in remission and healthy controls.

Methods

The IIM patients with active disease (included patients enrolled in repository corticotropin injection trial), in remission, and healthy controls were enrolled in this cross-sectional observational study. Serum concentrations of 51 cytokines/chemokines were obtained by utilising a bead-based multiplex cytokine assay (Luminex®). The myositis core set measures were obtained for all the patients. Cytokines with the best predictive ability to differentiate these clinical groups were assessed with three methods: 1) Least Absolute Shrinkage and Selection Operator modelling, 2) stepwise approach, and 3) logistic regression model.

Results

Twenty-one IIM patients with active disease, 11 IIM patients in remission and 10 healthy controls were enrolled. Myositis patients had elevated levels of chemokines that attract eosinophils (eotaxin) and dendritic cells, NK cells, cytotoxic T-cells and monocytes/macrophages (CXCL-9, IP-10), cytokines that drive T-helper 1 responses (TNF-a, lymphotoxin-a), matrix degrading enzymes (MMP-3 and -9), and IGFBP-2 compared to healthy controls. Myositis patients with active disease had higher levels of lymphotoxin-a, CXCL-9, MIP-1a, MIP-1b and MMP-3 than patients in remission.

Conclusion

This study demonstrated differences in cytokine profiles of IIM patients (active and inactive disease) compared to healthy controls and identified some cytokines that could potentially be used as biomarkers. Larger longitudinal studies are needed to validate our findings.

Key words

cytokine, myositis, biomarker

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Received on May 19, 2023; accepted in revised form on September 4, 2023.

The work should be attributed to the Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh, USA.

Funding: this study was funded by Mallinckrodt Pharmaceuticals.

Competing interests: C.V. Oddis has received clinical trial support for Rituximab trial, NIAMS, NIH (contract N01-AR-4-2273), study drug by Genentech. D.P. Ascherman has received research grant from Mallinckrodt Pharmaceuticals. R. Aggarwal has received research grants from Boehringer Ingelheim, Bristol-Myers Squibb, EMD Serono, Janssen, Mallinckrodt, Pfizer and Q32. He has received consulting fees from Actigraph, Alexion, ANI Pharmaceutical, Argenx, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, CabalettaBio, Capella, Corbus, CSL Behring, EMD Serono, Galapagos, Horizontal Therapeutics, I-Cell, Immunovant, Janssen, Kezar, Kyverna, Merck, Novartis, Nuvig Therapeutic, Octapharma, Pfizer, Regeneron, Roivant, Sanofi and Teva.
The other authors have declared no competing interests.

Introduction

Idiopathic inflammatory myopathies (IIM), also known as myositis, are a heterogenous group of systemic autoimmune diseases which include dermatomyositis (DM), and polymyositis (PM) (1). IIM is characterised by muscle weakness, but can also involve other organs causing rash, interstitial lung disease, and arthritis. Disease activity in myositis is primarily determined by physician assessment due to the lack of robust objective outcome measures. Furthermore, there are no currently available biomarkers to assist in predicting long term outcomes and treatment response in patients with IIM. Treatment involves glucocorticoids, intravenous immune globulins, and various immunosuppressive agents [methotrexate, azathioprine, tacrolimus, mycophenolate mofetil, repository corticotropin injection (RCI)], though there are a limited number of FDA approved therapies in IIM.

The pathogenesis of IIM is not clearly understood. Muscle biopsy in DM shows predominant perimysial and perivascular inflammation composed of CD4 (+) T cells, B cells, dendritic cells and macrophages, whereas polymyositis is predominantly associated with CD8 (+) cytotoxic T cell infiltration of endomysium surrounding non-necrotic muscle fibres (2). Deposition of membrane attack complex in capillary endothelial cells causes release of pro-inflammatory cytokines and expression of adhesion molecules that attract inflammatory cells in the perimysium (3, 4). Complement-mediated microangiopathy in DM with significantly reduced intramuscular capillary density leads to local ischaemia which could potentially explain the atrophy of perifascicular muscle fibres seen in histopathology (5). Healthy muscle fibres do not express MHC1, and acquisition of MHC1 expression in IIM allows muscle fibres to interact with T cells (6; 7). Unlike DM, invasion of healthy muscle fibres by CD8 (+) cytotoxic T cells and polarisation of perforin granules towards the muscle fibre surface suggest T-cell mediated cytotoxicity in PM (7, 8). These findings and the clonality of autoinvasive cytotoxic T cells suggest a common, but currently unknown, antigenic trigger on the myofibre surface (9, 10). Although the predominant cell type in both DM and PM is T cells, activated B cells may be playing important roles in disease pathogenesis given the increasing number of autoantibodies associated with myositis and the therapeutic benefit of intravenous immunoglobulin as well as rituximab therapies (11, 12). Overall, distinct histopathological findings associated with differential localisation of immune cells and the presence of different types of immune cells in muscle tissue suggest critical roles for cytokines and chemokines in IIM pathogenesis. Cytokines and chemokines are responsible for attracting immune cells and regulating leukocyte trafficking and activation, and could be produced by muscle fibres, macrophages, dendritic cells, fibroblasts, endothelial cells, and mast cells (13). Improved understanding of the role of cytokines in IIM could shed light on disease pathogenesis, facilitate the identification of biomarkers for prognostication, assist in monitoring of disease activity and treatment response, and potentially identify targets for treatment in IIM. However, studies investigating the role of cytokines in IIM are limited.

In this study, we therefore aimed to describe the serum cytokine profiles of patients with active and inactive IIM compared to healthy controls and to assess the correlations of these cytokines with clinical myositis outcome measures in order to define their potential as disease activity biomarkers.

Methods

Study design and participants

This was a cross-sectional study with three clinical groups enrolled prospectively: I) myositis patients with active disease, II) myositis patients in remission (*i.e.* inactive), and III) healthy controls. The active disease group included 10 patients enrolled in a 6-month open label clinical trial with Repository Corticotropin Injection (RCI, Acthar® Gel; Mallinckrodt Pharmaceuticals, NCT01906372) and 11 patients enrolled in an observational cohort with similar inclusion/exclusion criteria as the RCI trial (14). Inclusion

Table I. Baseline characteristics of the study participant groups including myositis patients with active disease, those who are in remission, and those with active, refractory disease enrolled in the RCI clinical trial, and healthy controls.

	Myositis patients with active disease on SOC (n=11)*	Myositis patients in the RCI clinical trial (n=10)*	Myositis patients in remission (n=11)	Healthy controls (n=10)
Age (± SD)	51.4 ± 20.9	51.4 ± 13.2	58.5 ± 15.9	46.6 ±11.4
Gender (M/F)	4/7	1/9	1/10	1/9
Diagnosis (PM/DM)	2/9	4/6	1/10	-
Disease duration (yrs)	0.7	1.9	3.9	-
Myositis Core Set Measures				
Manual muscle test (0-150)	125.6 [119-150]	118.5 [108.5-134.2]	150 [147.1-150]	-
HAQ (0-3)	1.2 [0.5-1.8]	1.5 [0.2-1.6]	0.7 [0-1.3]	-
Physician disease activity (0-10)	5 [3.7-5.7]	4.7 [2.6 – 6.5]	1.5 [0-3.5]	-
Patient disease activity (0-10)	5 [3-7]	5 [2 – 5.6]	5 [1-7.5]	-
Extra-muscular global disease activity (0-10)	1.5 [0.7-4.7]	2.1 [0.7 – 3.6]	0.6 [0-1.5]	-
Creatine kinase level (IU/L)	75 [51-213]	386 [66 – 2765]	162 [7.5-306]	-

SOC: standard of care; RCI: repository corticotropin injection; HAQ: Health Assessment Questionnaire.
Data is presented as median [interquartile range].
*No significant differences were observed between patients with active disease on SOC and those in the RCI clinical trial for all the variables: age (p=0.9), MMT (p=0.1), HAQ (p=1), physician disease activity (p=0.8), patient disease activity (p=0.6), extra-muscular global disease activity (p=0.8), and creatine kinase levels (p=0.2).

criteria for the RCI trial were defined as refractory and active disease based on failing an adequate glucocorticoid trial (≥2 months of high doses (0.75–1 mg/kg) or intolerance to such therapy) and/or ≥1 conventional immunosuppressive agent at near maximal doses for ≥3 months (14). Active disease in the prospective cohort was determined by the treating physician. Remission was defined as ≥6 months continuous period with no evidence of disease activity (14). All patients fulfilled the 2017 ACR/EULAR myositis classification criteria for probable/definite DM/PM and European Neuromuscular Centre for immune-mediated necrotising myopathy (IMNM) (15, 16).

Clinical measures

IIM patients in all four groups had six myositis core set measures collected including physician-reported global disease activity (MDGD), patient reported global disease activity (PTGD), extra muscular global disease activity (EXGD), manual muscle testing (MMT), Health Assessment Questionnaire (HAQ), and muscle enzymes (Creatine Kinase (CK)). In addition, cutaneous, pulmonary and muscle disease activity were collected using the Myositis Disease Activity Assessment Tool. This study was approved by the Institutional Review Board of the University of Pittsburgh Medical Centre (IRB# PRO16100125). Informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Luminex assays

Venous blood samples were obtained from all the participants, and serum concentrations (pg/ml for all except angiotensinogen which was ng/ml) of 51 cytokines/chemokines were assessed using a bead-based multiplex cytokine assay (Millipore Hu High Sensitivity, 18-plex [Cat# HSTCMAG-28SK-18], Millipore Cytokine panel 1, 14-plex [Cat# HCYTOMAG-60K-14], Millipore Hu Neurological Disorders Panel 3, 1-plex [Cat# HND3MAG-39K-01], Luminex Human Magnetic Assay, 4-plex [Cat# LXSAHM-04], 12-plex [Cat# LXSAHM-12], and 3-plex [Cat# LXSAHM-03]). The cytokines included in this study were determined based on previously published literature on this topic in IIM and juvenile myositis, and included angiopoietin-2, angiotensinogen, B-cell activating factor (BAFF), C-C motif ligand 5 (CCL5), CD23, CD25, coagulation factor XIV/protein C, CXC motif chemokine ligand 9 (CXCL9), fractalkine, IFN-α2, IFNβ, IFNγ, Insulin-like growth factor binding protein 2 (IGFBP-2), IL-1α, IL-1β, IL-1Ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17a, IL-18, IL23, insulin, interferon-inducible protein 10 (IP-10), interferon-inducible T cell alpha chemoattractant (ITAC), leptin, lymphotoxin-a, monocyte chemoattract protein 1 (MCP-1), MCP-3, macrophage derived chemokine (MDC), macrophage inflammatory protein 1 alpha (MIP-1α), MIP-1β, matrix metalloproteinase 3 (MMP-3), MMP-7, MMP-9, MMP-12, progranulin, resistin, transforming growth factor alpha (TGF-α), tumour necrosis factor alpha (TNF-α), and TNF-β.

Statistical analyses

Comparisons of IIM patients *versus* healthy controls and patients with active disease *versus* inactive disease was performed with the Mann-Whitney U test and calculation of False Discovery Rate (FDR). Cytokines with the best predictive ability to differentiate these clinical groups were assessed with three methods: 1) Least Absolute Shrinkage and Selection Operator (LASSO) modelling, 2) a stepwise approach, and 3) logistic regression model.

The cytokines that were found to have $p<0.1$ via Mann Whitney U testing were included in the multivariable logistic regression analysis. The cytokines that were found to be significant ($p<0.05$) in multivariate logistic regression analysis were included together in the final multivariable logistic regression analyses. Each model was controlled for age,

Table II. The median levels of cytokines that were found to be at higher levels in myositis patients with active and inactive disease based on Mann-Whitney U test and false discovery rate (FDR) results.

	Active myositis (n=21)	Inactive myositis (n=11)	*p*-value	Healthy control (n=10)	All myositis (n=33)	*p*-value
Angiotensinogen*	893.2 [291.1-3227]	1283 [577.2-4126]	0.89	2086 [372.4 - 5810]	1151 [311.5-3274]	0.37
Angiopoietin-2	2973 [1903-3640]	2012 [1296-3411]	0.14	2134 [1366-2388]	2406 [1862-3593]	0.10
CD-25	713.2 [479.8-1266]	756.8 [476.8-896.5]	0.65	557.8 [423.1-608.9]	726.3 [480.7-1032]	0.07
CXCL9‡‖¶	2394 [2124-2806]	2089 [1225-2148]	0.01	1427 [1286-1599]	2198 [1668-2573]	0.048
Eotaxin*‡	235.7 [170.3-304.2]	210.5 [152.5-263.5]	0.29	98.9 [65.9-158.1]	232.1 [164.9-276]	0.0001
IFN-γ	18.3 [13.4-36.7]	14.3 [11.9-21.3]	0.27	24.3 [6.6-26.5]	18.2 [12.2-25.3]	0.93
IGFBP-2*‡	498129 [284887-793760]	472030 [239488-637574]	0.54	114824 [54136-332017]	485079 [250879-684668]	0.0007
IL-1α	276.2 [58.9-555.3]	219.9 [142.2-521.8]	0.78	41.1 [14.8 - 223]	259.2 [121.7-555.3]	0.01
IL-1b	1.4 [0.8-3.0]	1.8 [1.1-2.6]	0.39	1.1 [0.6-2.8]	1.6 [1.0-3.0]	0.35
IL-1Rα	115.2 [55-177]	73.5 [46.4-142]	0.44	31.5 [16.2-82.7]	100 [49.2-156.8]	0.006
IL-3	0.2 [0.1-0.2]	0.2 [0.2-0.3]	1	0.1 [0.1-0.3]	0.2 [0.1-0.2]	0.21
IL-4	159.2 [54.2-608.5]	229.5 [98.6-782.1]	0.29	69.3 [22.9-189.7]	208.6 [66.9-610.0]	0.005
IL-5	8.5 [3.7-18.7]	6.9 [5.4-19.9]	0.46	6.5 [2.0-10.0]	7.7 [4.9-19.0]	0.07
IL-6	19.6 [7.0 - 55.1]	29.1 [10.2-76.6]	0.29	15.4 [2.2-21.7]	24.2 [8.0-58.2]	0.009
IL-8*§	31.7 [20.0-76.6]	74.9 [23.3-96.4]	0.14	17.8 [10.2-585.2]	35.1 [20.4-87]	0.07
IL-9†	18.3 [11.3-54.9]	10.4 [4.8-36.0]	0.82	3.5 [3.5-3.5]	17.5 [6.5-37.3]	0.001
IL-13	14.2 [5.3-39.1]	19.8 [9.7-65.2]	0.31	11.3 [1.9-18.2]	17.1 [6.8-45.9]	0.09
IL-15	7.2 [4.3-13.9]	7.1 [4.7-9.8]	0.77	7.1 [6.6-7.6]	7.2 [4.4-12.3]	0.0002
IL-17A	17.5 [9.9-30.1]	16.5 [8.1-17.7]	0.35	19.7 [7.0 - 23.0]	16.9 [8.4-25.0]	0.96
IP-10†‡	679 [362.1-1991.0]	323 [181.7-992.6]	0.13	192 [145.6-252]	539.2 [272.9-1378]	0.0005
Leptin§	10079 [4194-25311]	29537 [3394-38626]	0.46	14875 [4093-31605]	11047 [4093-32273]	0.98
Lymphotoxin-α*‡§¶	8.8 [4.3-10.7]	4.4 [2.1-6.4]	0.002	2.5 [2.0-3.4]	6.8 [3.3-10.1]	0.0008
MDC	1077 [629.7-1584]	1437 [885-1683]	0.59	1677 [1445-1934]	1322 [670-1595]	0.02
MIP-1β§¶	34.6 [23.8-49.8]	21.3 [9.7-33.7]	0.04	31.5 [22.2-40.8]	32.5 [17.7-44.3]	0.91
MMP-3‡¶	34167 [17643-41948]	23524 [8034-24266]	0.01	10440 [8701-12528]	25691 [13883-37732]	0.002
MMP-9†	308540 [176110-475386]	308980 [171414-439449]	0.98	106816 [54136-305201]	308760 [176888-465813]	0.03
Progranulin§‖	44833 [36517-57004]	47183 [44274-62850]	0.18	46214 [42988-62554]	46013 [40287-57457]	0.91
Resistin§‖	13863 [8878-20409]	16360 [7473-22812]	0.15	12867 [11259-14992]	13863 [8878-20456]	0.68
TNF-α	9.5 [5.8-10.9]	7.8 [6.2-11.8]	0.78	6.7 [6.3-7.8]	9.2 [5.9-11.5]	0.06
TNF-β*	539.9 [333.9-881.3]	266.8 [26.2-736-1]	0.47	64.4 [8.7-169.1]	451.7 [152-785.4]	0.009

Cytokines/chemokines are listed in alphabetical order and with unit as pg/ml, data is presented as median [interquartile range].
*Cytokines predictive of myositis *vs*. control using LASSO modelling.
†Cytokines predictive of myositis *vs*. controls using stepwise approach.
‡Cytokines that differentiate cases *vs*. controls using regression model controlling for age and gender.
§Cytokines predictive of active *vs*. remission using LASSO modelling.
‖Cytokines predictive of active *vs*. remission using stepwise approach.
¶Cytokines that differentiate active disease *vs*. remission using a regression model controlling for age and gender.

gender, and diagnosis, as permitted by sample size. Spearman correlations were used to assess correlations between cytokine levels and myositis outcome measures. Statistical analysis was performed with Stata (adaptive LASSO with lowest lambda to select the final variables) and R (glm, and stepAIC in MASS package) softwares. Pathway analysis using GeneOntology was performed to identify pathways associated with aberrantly expressed cytokines.

Results

Study participants

The baseline demographics and clinical characteristics of all four groups are presented in Table I. The active disease group was comprised of 10 patients (4 PM-IMNM/6 DM) from the RCI clinical trial and 11 patients (2 PM-IMNM/9 DM) from a separate observational cohort. The inactive disease group included 11 patients (1 PM-IMNM/10 DM). All groups were age and gender matched to the active group from the RCI clinical trial. No significant differences in myositis core set measures were found between patients with active disease enrolled in the trial and those from the prospective observational cohort; therefore, these groups were combined in this study as "active disease group".

Differences in cytokines between healthy controls and myositis patients

The cytokines that were found to be higher and lower in myositis patients compared to healthy controls are shown in Table II (based on FDR <0.20). Cytokines and chemokines with predictive ability to differentiate myositis patients (active and inactive disease) from healthy controls using LASSO regression modelling were lymphotoxin-α (coefficient 22.60), TNF-β (17.76), eotaxin (4.60), IGFBP-2 (1.88), angiotensinogen (-1.87), and IL-8 (-1.07). The best predictive model capable of differentiating myositis patients from healthy controls included a combination of IL-2, IL-9, IP-10, and MMP-9 based on stepwise approach (AUC=1 in internal validation set). Logistic regression model controlled for age and gender revealed IGFBP-2, lymphotoxin-α, eotaxin, and IP-10 as cytokines that significantly predict cases *versus* controls ($p<0.05$). Pathways associated with increased levels of these cytokines included those linked to chronic inflammatory response, regulation of IL-1

mediated signalling, myoblast fusion, and tyrosine phosphorylation of STAT protein.

Differences in cytokines between active myositis patients vs. myositis patients in remission

Based on LASSO regression modelling, cytokines and chemokines that effectively differentiated myositis patients with active disease from those in remission were lymphotoxin-α (coefficient 4.81), insulin (1.54), MIP-1β (1.37), IFN-β (0.44), MCP-1 (0.20), resistin (-1.62), leptin (-1.33), progranulin (-0.52), and IL-8 (-0.41). Predictive models which best differentiate active myositis *versus* inactive myositis included a combination of IFN-β, TGF-α, CXCL9, IL-1α, resistin, progranulin, and IL-18 based on stepwise approach. Logistic regression model controlled for age and gender revealed lymphotoxin-α, CXCL9, MIP-1β, and MMP-3 as cytokines that significantly predict active disease *versus* remission ($p < 0.05$). Taken together, the cytokines that repeatedly shown to be significantly different between patients and controls *and* between patients with active *versus* inactive disease were lymphotoxin-α, CXCL-9, MMP-3, MCP-1, and IL-8 (Fig. 1).

Differences in levels of promising cytokines in subgroups of myositis patients

Generally, no significant differences were found in cytokine levels between patients with DM (n=25) and PM-IMNM (n=7), with the exception of IL-18 (median 374.5 *vs*. 255.8 pg/ml) and ITAC (101.4 *vs*. 44 pg/ml) which were higher in DM and TGF-α (median 4.1 *vs*. 13.1 pg/ml) which was higher in PM-IMNM.

Correlations between cytokine levels and clinical outcome measures in myositis patients

No significant correlations were found between myositis core set measures and the majority of cytokines that were analysed (Supplementary Fig. S1, Suppl. Table S1). The most notable correlations (moderate to strong correlations) included correlations between manual muscle testing and CCL5, protein C, MMP7, resistin and IL-3; cutaneous disease activity and IP-10 and IL-2; extra-muscular global disease activity and CD25, MMP3, BAFF, CXCL9, lymphotoxin-α, IL-13, IL-17a, and IL-23; muscle disease activity and leptin; and physician global disease activity and CXCL9 and MIP-1a (Rho ranging from 0.40 to 0.61).

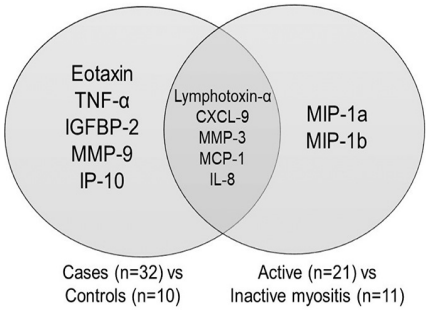

Fig. 1. The cytokines that showed significant predictive ability to differentiate the clinical groups based on all the three methods utilised in the study.

Discussion

Through detailed multiplex ELISA analysis of serum samples, our study demonstrates differences in cytokine profiles of patients with IIM (active and inactive disease) compared to healthy controls. Signalling mediators capable of distinguishing different IIM states from controls included CXCL9, IP-10, eotaxin and MCP-1.

As IFN-inducible chemokines, CXCL9 and IP-10 (or CXCL10) were significantly higher in patients with myositis compared to healthy controls. These results were consistent with previous studies showing elevated levels of IP-10 in patients JDM, DM, and EJ (+) antisynthetase syndrome compared to controls (17-20). In one study, IP-10 levels had decreased and reached levels close to healthy controls after 5 months of treatment (19). In another study by Uruha *et al*. IP-10 levels were significantly higher in patients with IIM compared to patients with hereditary muscular dystrophies--with levels >650 pg/ml having a sensitivity and specificity of 91% and 90%, respectively (21). IP-10 also showed correlations with global disease activity, manual muscle strength testing, and muscle disease activity (22). In our study, patients with active disease also had an elevated median level of IP-10 (679 pg/ml), which was higher than patients with inactive disease (323 pg/ml). Furthermore, CXCL-9 was able to significantly predict active versus inactive disease state.

Coupled with their ability to differentiate IIM patients from controls and distinguish activity states of IIM, the observed correlations with clinical disease activity suggest that CXCL9 and IP10 represent promising biomarkers in IIM. Both CXCL9 and IP-10 bind to CXCR3 as receptors that are preferentially expressed on activated Th1 cells (as well as tissue resident macrophages and natural killer cells) (23). Studies with muscle biopsies from PM and DM have shown strong expression of CXCR3 on T cells as well as high levels of CXCL9 and IP-10 in muscle tissue and abundant expression of IP-10 on macrophages and T cells that surround and invade muscle fibres in PM or infiltrate the perimysium in DM (24, 25). Thus, elevated levels of CXCL9 and IP10 in IIM support a disease paradigm involving recruitment and activation of Th1 cells in actively inflamed muscle tissue. Patients with IIM also had significantly higher levels of MCP-1 (a type I IFN-regulated chemokine) and eotaxin compared to healthy controls. These results were consistent with previous studies showing elevated levels of MCP-1 and eotaxin in patients with JDM and IIM compared to controls (17, 18, 21, 22). Interestingly, MCP-1 and eotaxin bind to the same receptor, CCR2. An *in vitro* study has demonstrated that eotaxin displaces MCP-1 in a concentration dependent manner and inhibits MCP-1-induced chemotaxis and enzyme release from macrophages (26). Upregulation of MCP-1 expression in endothelial and smooth muscle cells along with elevated circulating levels of both MCP-1 in IIM suggests a potential role for MCP-1 in recruitment of monocytes, memory T cells, and plasmacytoid dendritic cells into muscle tissue. However, these effects may be counterbalanced by elevated levels of the eosinophil chemoattractant eotaxin, which limits propagation of inflammation (27). Even though eosinophils are not one of the dominant cell types in

IIM, the number of eosinophils is significantly higher in muscle tissue of patients with IIM compared to normal controls (28). Despite these provocative findings implicating MCP-1 and eotaxin in the disease processes of IIM, our study did not show significant differences in MCP-1 and eotaxin levels between patients with active and inactive disease, similar to a previous study (17). This could be due to the small number of patients with active and inactive disease, variable disease duration, or likely imperfect classification of activity determined solely by physician assessment. Nonetheless, further studies are required to understand the role of these cytokines in regulation of inflammation in IIM and their potential as therapeutic targets.

There is growing body of evidence to suggest a substantial role for type I IFN (IFN-α, IFN-β) in disease pathogenesis, specifically in DM where transcripts and proteins representing type I IFN induced pathways are increased in muscle fibres and blood (29). A Type II IFN chemokine signature (calculated based on IFN-inducible T cell α chemoattractant (ITAC), IP-10, MCP-1, MCP-2) was also higher in DM patients than controls and correlated with disease activity (22). Unfortunately, in our study, the measured levels of IFN-α2 and IFN-β for most samples were below the detection limit of the Luminex assay and therefore could not be assessed due to low sensitivity of the technique. Although there was no overall difference in the levels of type I and II IFNs, responsiveness to these cytokines may differ between controls vs myositis and active *versus* inactive patients. Future studies assessing the expression level of receptors for type I and II IFNs in IIM are therefore required. Similar to previous studies, IFN-γ levels were not significantly different between patients and controls or between patients with active and inactive disease, despite the increase in IFNγ-inducible chemokines CXCL9 and IP-10 (17, 18). However, one study with treatment naive patients demonstrated elevated IF~N-γ levels in active myositis that decreased to the levels of healthy controls following treatment (27). Our study included patients with established disease who were already on treatment, potentially explaining the conflicting results with these studies.

Both IL-1α and IL-1Ra were significantly higher in patients with myositis than healthy controls, whereas IL-1β levels were similar between patients and controls. Previous studies showed conflicting results for IL-1α, IL-1β, and IL-1Ra levels between patients and controls, with some showing higher levels of IL-1β and IL-1Ra in patients and others showing no significant difference between groups (17, 18, 30). Notably, decreases in skeletal muscle expression and serum levels of IL-1α and IL-1Ra with therapy were previously demonstrated in several studies (30, 31). Thus, discordance in results of these studies could be due to heterogeneity of the study cohorts in regards to treatment history and disease activity.

IGFBP-2 was also significantly higher in patients with myositis than in healthy controls. IGFBPs are transport proteins for insulin-like growth factors that have recently attracted attention as potential disease activity biomarkers in several autoimmune diseases such as lupus nephritis and systemic sclerosis (32, 33). Insulin-like growth factor-independent actions of IGFBPs, including proliferation of effector lymphocytes and suppression of T regulatory cells, may suggest a role for these mediators in the IIM disease process, but further studies are required to better understand the mechanism of action of these proteins (34).

In a previous study by Bilgic *et al*., IL-6 was suggested as a candidate disease activity marker due to its correlations with global disease activity (22). IL-6 levels were shown to strongly correlate with type I IFN chemokine score, leading the authors to suggest a specific co-regulation of IFN and IL-6 production in patients with DM (22). In our study, although the patients with myositis had significantly higher levels of IL-6 compared to controls, there was no significant difference between patients with active disease vs remission, and IL-6 levels did not correlate with global disease activity measures. Similar to our study, some studies also showed increased levels of IL-6 in myositis patients, whereas others showed comparable levels between myositis patients and controls (18, 19, 27, 30). These conflicting results could be due to heterogeneity of the cohorts in regards to DM and PM, as well as the small sample sizes of these studies. These discrepancies likely reflect differences in disease activity or treatment regimens, as the study by Bilgic *et al*. demonstrating higher levels of IL-6 levels in DM included a large number of treatment-naïve patients.

Finally, both TNF-α and lymphotoxin-α (or TNF-β) levels were significantly higher in the serum of patients with myositis than in healthy controls, and lymphotoxin-α levels were significantly higher in patients with active disease compared to patients with inactive disease. Although previous studies did not show any difference in *serum* levels of TNF-α between myositis patients and controls (18, 30), other studies performed in PM/DM muscle samples showed increased TNF-α expression in muscle fibres and inflammatory infiltrates (13), highlighting the more general issue that serum levels of cytokines/chemokines may not always reflect the cytokine milieu of a specific tissue compartment such as muscle. In fact, the observed upregulation of TNF-α in muscle prompted clinical trials of TNF inhibitors in IIM, which did not result in significant clinical improvement (35, 36) for reasons that could include limited tissue penetration of these medications as well as relative expression of TNF-α receptor subtypes in muscle.

Limitations of our study include the limited sample size, cross sectional design, and heterogeneity of our cohort that is likely underpowered to detect differences between active *versus* inactive disease states or between PM *versus* DM. Although provocative, our conclusions are therefore limited and will require more extensive evaluation in larger studies. Furthermore, the patients included in our study had established disease on standard of care treatment regimens that often included glucocorticoids, which may affect the results of cytokine levels as discussed above. More generally, this study was not pow-

ered to control for different anti-inflammatory therapeutic regimens that could skew cytokine profiles. Nevertheless, our results, which suggest a number of cytokines/chemokines that may contribute to myositis pathogenesis and also serve as useful biomarkers of disease activity, clearly provide the foundation for future studies in this arena.

Conclusion

We identified several potentially promising cytokines and chemokines that can be used to distinguish IIM disease states and should be further studied for their diagnostic and prognostic utility in future studies.

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