

Using peripheral blood immune signatures to stratify patients with adult and juvenile inflammatory myopathies

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ABSTRACT:

Objective: The inflammatory idiopathic myopathies (IIM) are a group of rare autoimmune diseases defined by muscle weakness and characterised by pro-inflammatory infiltrates in muscle. Little is known about the immunological profile in peripheral blood of these patients and how this relates to IIM subtypes. This study aimed to stratify adult and juvenile-onset IIM patients according to immune cell profile.

Methods: Peripheral blood mononuclear cells from 44 patients with adult myositis (AM), 15 adolescent-onset juvenile dermatomyositis (a-JDM), and 40 age-matched healthy controls were analysed by flow cytometry to quantify 33 immune cell subsets. AM patients were grouped according to myositis subtype; dermatomyositis (DM) and polymyositis (PM), and also autoantibody specificity. Disease activity was determined by the myositis disease activity assessment tool and clinicians' decision on treatment.

Results: Unique immune signatures were identified for DM, PM and a-JDM compared to healthy controls. DM patients had a T-cell signature comprising increased CD4⁺ and TH17 cell frequencies and increased immune cell expression of IL-6. PM patients had a B-cell signature with reduced memory B-cells. A-JDM had decreased naïve B-cells and increased CD4⁺T-cells. All patient groups had decreased CD8⁺central memory T-cell frequencies. The distinct immune signatures were also seen when AM patients were stratified according to auto-antibody expression; patients with anti-synthetase-antibodies had reduced memory B-cells and patients with autoimmune rheumatic disease overlap had an elevated Th17 profile.

Conclusion: Unique immune signatures were associated with adult versus juvenile disease. The Th17 signature in DM patients supports the potential use of IL-17A inhibitors in treatment of IIMs.

Keywords: Myositis, immune signature, Th17, interleukin-6

KEY MESSAGES

- Unique peripheral blood immune signatures identify sub-groups of Inflammatory Idiopathic Myopathies (IIM).
- Adult dermatomyositis (ADM) patients have a Th17 signature and elevated IL-6 production.
- Immune stratification could identify adult dermatomyositis (ADM) patients that would benefit from anti-IL-17 or anti-IL-6.

INTRODUCTION

The Inflammatory Idiopathic Myopathies (IIM) are a group of rare myopathic autoimmune diseases diagnosed in both adults and children. Patients present with a variety of features including proximal muscle weakness and, in the case of dermatomyositis (DM), skin changes. Up to 80% of patients have unique serum autoantibody profiles with associated clinical features(1). However, although immunohistochemical analysis of muscle tissue from IIM patients has identified immune cell infiltrate and the expression of pro-inflammatory cytokines(2, 3), very little is known about the peripheral immunological profile in juvenile and adult patient groups(4).

The adaptive immune system is implicated in the pathogenesis of IIM(4) and distinct patterns of immune cell accumulation have been described in muscle and skin biopsies from polymyositis (PM) and DM patients(5, 6). The endomysial cellular infiltrates most commonly detected in PM, constitute of CD8+ T cells, CD4+ T cells, dendritic cells (DCs) and macrophages. These cells localise around the muscle fibres. In DM, the perivascular infiltrates consist of CD4+ T cells, DCs, macrophages and B cells. Unlike PM, these cells are found around the inflamed blood vessels (7).

T cells are dysfunctional in the context of IIM. CD4+CD25+FOXP3+ regulatory T cells were shown to lose their ability to regulate the immune system in patients with active JDM (8, 9) and pro-inflammatory T helper-17 (Th17) cells have been found in tissue biopsies from IIM patients and were positively correlated with disease activity (7, 9-11). Conversely, B cells have not been thought to be as vital as T cells in the pathogenesis of IIM although they do play an important role. Not only are B cells found in the infiltrates of muscle tissue in DM patients, there is a high detection of myositis-associated (MSA) and specific (MSS) autoantibodies (12-14). The expression of B cell activating factor (BAFF) has been associated with anti-Jo-1 antibodies in DM muscle tissue, specifically in the perifascicular area (15). Recently we have shown that immature B cells are expanded in juvenile dermatomyositis and are skewed towards a pro-inflammatory phenotype by Toll-Like Receptor 7 and Interferon- α (16).

Most studies on IIM have focused on the inflammatory milieu found within the tissue biopsy. The biopsy is a key diagnostic tool, but is rarely repeated past initial presentation, thus little is known about the histological changes during the disease course. On the other hand little is known about the peripheral immunological profile and how this changes with disease activity. This study investigated the peripheral blood immunological profile from adult and juvenile IIM patients.

MATERIALS AND METHODS:

Patients and controls

Peripheral blood mononuclear cells (PBMC) from 44 adult myositis (AM) patients (including dermatomyositis (DM) and polymyositis (PM)), 15 adolescent-onset juvenile dermatomyositis (a-JDM) patients, 25 age, sex and ethnicity-matched adult healthy controls (AHC), and 15 age, sex and ethnicity-matched teenage healthy controls (THC) were collected over a period of two years and were stored frozen in liquid nitrogen for a maximum time of one year (as previously recorded (16) at the Centre of Adolescent Rheumatology University College London Biobank(16). Participants were recruited after obtaining written informed consent with appropriate ethical approval (REC11/0101). Demographic and clinical information for patients and controls are shown in Table 1. A-JDM patients met Bohan and Peter criteria for probable or definite disease(17). Patients were diagnosed on the basis of their clinical features, serum creatine kinase, electromyogram, magnetic resonance imaging and muscle biopsy results. Myositis-associated and specific autoantibody specificities were recorded when performed as part of routine diagnostic procedure as part of the extended myositis autoantibody panel (Table-2).Disease activity was assessed using the myositis disease activity assessment tool (MITAX)(18, 19) and/or the clinicians' treatment decision (increase, decrease or no change in treatment). A-JDM patient disease activity was measured by the manual muscle test (MMT8)(20). To calculate the MITAX score; in each of the different organs and systems assessed a patient deemed sufficiently active to require at least 20mg of steroids +/- an immunosuppressive drug is assessed as a grade A (12 points); a patient with moderately active disease requiring a lesser amount of steroid/immunosuppressive is assessed as a B (8 points); a patient with mild disease requiring topical treatments only or Hydroxychloroquine/non-steroidal anti-inflammatories is assessed as a C (1 point); a patient whose disease was once active in the individual organ system but is no longer active is assessed as a D (0 points); and a patient whose organ systems were never previously involved is assessed as an E (0 points). Patients were categorised into; active, mildly active, remission on-treatment and remission off-treatment.

Flow cytometry

PBMCs were analysed by flow cytometry after staining for viability (Median average – AM (95.30%), AHC (94.35%), JDM (86.70%), THC (85.20%) Supplementary Figure 2 and gating strategy Supplementary Figure 3. Surface staining *ex-vivo* with directly conjugated antibodies and intracellular cytokines according to previously defined protocols after 4hr culture with PMA, ionomycin and Golgi plug(16) (Supplementary Table 1). Data were collected on an LSRII flow cytometer (BD Pharmingen) using FACS Diva software and analysed by Flowjo (Treestar).

Statistical analysis

Statistical analysis was performed in GraphPad Prism 6 and SPSS (V.24). Unpaired student t-tests were used to compare *ex-vivo* cell populations from patients and controls. Fold change was calculated to determine the directional change in the mean average. Multiple comparisons were made comparing each cell population using multiple *t* tests with Holm-Sidak method of correction for multiple comparisons or one-way ANOVA and Tukey's multiple comparison test. MultiExperiment Viewer (MeV) was used to produce heat maps and perform hierarchical clustering.

RESULTS:

No significant difference in demographic profile was identified between IIM patient groups

Comparison of the clinical and demographic characteristics of AM and a-JDM patients are shown in Tables 1 and 2. The AM cohort included patients with, DM (n=19), PM (n=9), DM with cancer (n=4), DM (n=7) and PM (n=5) with overlap autoimmune rheumatic diseases (e.g. systemic lupus erythematosus, scleroderma and rheumatoid arthritis).

Myositis-associated and myositis-specific autoantibodies were identified in the majority of AM and a-JDM patients (n=33; 84.1% and n=12, 80% respectively) (no results available for 7 AM and 3 a-JDM patients). Patients with AM were characterised by a wide range of autoantibodies including anti-synthetase (anti-Jo-1 and anti-PL-12) antibodies (n=7); patients with autoimmune rheumatic disease overlap (anti-Ro, anti-PmSCL and anti-RNP) antibodies (n=9); cancer associated (anti-NXP2 and anti-TIF1 γ) group (n=4), and severe skin (anti-Mi2) group (n=3). Eleven patients had multiple autoantibodies detected, of which 8 were positive for anti-Ro in addition to a second antibody. Unfortunately, complete data were not available for the a-JDM cohort.

AM patients were treated with a broader spectrum of non-biologic drugs including tacrolimus, cyclosporine and intravenous immunoglobulin-G (IVIG) compared to a-JDM patients. Rituximab was the only biologic used in the AM cohort (n=7, 15.9%), whereas infliximab (n=2, 13.3%), adalimumab (n=1, 6.7%) and rituximab (n=1, 6.7%) were used in the a-JDM cohort.

Overall analysis of these IIM patient subgroups showed that patients with DM with cancer had elevated ESR (p=0.03) and higher MITAX score (although this was not significant). No other significant differences were identified between groups in terms of clinical and disease features, including skin disease (Tables 1 and 2 and Supplementary Figure 1).

Distinct immune signatures characterise adult and juvenile DM

In order to assess whether the different patient groups were characterised by unique immune cell profiles in depth immune phenotyping was performed including analysis

of 33 PBMC populations (Supplementary Figure 3 and Supplementary Table 2 for gating strategies) comprising T-cell, B-cell and monocyte sub-populations. Significant changes in immune profile between AM patients and age-matched healthy controls (Figure 1A and B, Supplementary Table 3) were identified.

Stratification of IIM patients revealed unique immune signatures associated with disease subgroups including increased Th17 and regulatory T cell (Treg) populations and decreased memory B-cells (early(e)BM5) in patients with ADM compared to adult healthy controls (AHC) (Figure 1C) and decreased CD27⁺ B-cells and CD8⁺ CM T-cells in APM patients (Figure 1D). Patients with a-JDM were characterised by significantly decreased naïve B-cells (BM2), total CD8⁺ and CD8⁺ central memory (CM) and natural killer (NK) cell populations and increased total CD4⁺ T-cells in a-JDM patients compared to teenage healthy controls (THC) (Figure 1E). Furthermore, when ADM and a-JDM patient groups were compared directly, ADM patients retained their unique elevated Th17/Treg and decreased memory B-cells signature but also had reduced CD8⁺ naïve and intermediate monocyte populations compared to a-JDM patients (Figure 1F). Age did not influence the disease-associated profiles when adult and teenage healthy controls were compared (Figure 1G). Refer to Supplementary Table 3 for specific values of significant differences.

Distinct immune signatures were associated with myositis associated and specific autoantibody profiles.

Complex patterns of myositis associated and specific autoantibodies have been widely linked to IIM subgroups and distinct clinical features(21) (22). Correlation of the immune phenotype of AM patients with their autoantibody status revealed patients with anti-synthetase (anti-Jo-1 and anti-PI-12) antibodies (n=7) had decreased CD27⁺B-cells compared to AHC (Figure 2A) and patients with autoimmune rheumatic disease overlap (anti-Ro, anti-PmSCL and anti-RNP) antibodies (n=9) demonstrated a T-cell signature with increased Th17 and decreased CD8⁺CM populations (Figure 2B). The cancer associated (anti-NXP2 and anti-TIF1 γ) group (n=4) showed increased total monocyte, intermediate monocyte and plasmablast (BM3-4) B-cell populations, but decreased total T-cells (Figure 2C). There were no significant phenotypic changes when the severe skin (anti-Mi2) group

(n=3) was compared to AHC (Figure 2D), or in patients with multiple autoantibody profiles or patients negative for autoantibodies (data not shown) compared to healthy controls. Thus, stratifying according to autoantibody profile also revealed specific immune phenotype profiles, in particular in the anti-synthetase and overlap subgroups.

Disease activity did not drive increased Th17 cell frequency in AM patients

The AM cohort was further assessed according to disease activity. Four disease activity groups were identified using the clinicians decision on treatment criteria; active (n=4), mildly active (n=9), remission on-treatment (n=12) and remission off-treatment (n=5). The immune profile of AM patients in remission did have altered immune signatures; patients in remission off-treatment were characterised by increased non-classical monocytes and naive B-cell populations and decreased memory CD27⁺B-cells (Figure 2E); while Th17 cells were elevated in patients in remission on-treatment (Figure 2F), potentially reflecting immune changes that occur in AM that continue into remission and are no longer dampened by treatment. However, the immune profile of patients with active or mildly active disease was not significantly altered compared to AHCs (n=25) (Figure 2G and 2H).

Increased Th17 cells were associated with an IL-6 signature in AM patients

Notably, IL-6 is detected at high levels in the serum and muscle of IIM patients and has been linked to active disease (23). Therefore peripheral blood immune cell activation (measured by CD69 expression) and IL-6 production (detected by intracellular staining) was assessed (Supplementary Figure 4 for gating strategies). AM but not a-JDM patients had a significant increase in both immune cell activation and IL-6 production compared with healthy individuals (Supplementary Figure 5 A-C and Figure 3A-C). Moreover, when AM patient subgroups were considered the IL-6 signature was only seen in the ADM patients (Figure 3D&E). Furthermore, a significant positive correlation was observed between Th17 expansion and T-cell activation (measured by CD69 expression) and IL-6 production by memory B-cells in AM patients (Figure 3F). No relationship was observed between IL-6 and Th17 expression and MITAX score (Supplementary Figure 6).

Thus unique immune signatures were identified in ADM, APM and a-JDM patient subgroups. All patients had decreased CD8⁺ CM cell frequencies. ADM patients had a T-cell signature with an increase in total CD4⁺ T-cells and Th17 cells, as well as increased pan-immune cell IL-6 production and immune cell activation. APM patients had a B-cell signature with a decreased memory B-cells and did not produce IL-6 (Figure 4). In contrast to ADM, a-JDM patients did not have a Th17/IL-6 signature and were associated with increased B-cell memory cell frequencies compared to THCs.

DISCUSSION

This study is the first to identify novel immune cell signatures in a heterogeneous group of patients with the very rare disease IIM; distinct immune-phenotypes were identified between disease subtypes, ages and disease activity. Specifically, ADM but not APM patients were associated with expansion of Th17 cells associated with elevated IL-6 expression, but all patient groups had reduced CD8⁺CM T-cell frequencies. Previous studies in peripheral blood have highlighted changes in total immune cell populations including monocytes, T and B lymphocytes, but have not correlated changes across a large number of different lymphocyte sub-populations (24).

Our aim was to report unique peripheral blood immune signatures that distinguished each subtype of AM and a-JDM. Our findings suggest that ADM is a more IL-6 and IL-17 driven disease compared to APM or a-JDM. Th17 T-cells selectively produce IL-17 over interferon (IFN)- γ and depend on IL-6 amongst other cytokines for their differentiation. The Th17 pathway plays a critical role in the induction and maintenance of chronic inflammation and autoimmunity and Th17 cells have been found in the muscle tissue of IIM patients(25). IL-17 in conjunction with IL-1 and TNF- α increases the expression of MHC class I on muscle cells, relevant in the context of IIM as there is overexpression of MHC class I(26). The induction of IL-6 by IL-17 may also be involved in the dysfunctional repair of inflamed muscle(25).

IL-17 inhibitors have been considered to be potentially beneficial for use in IIM patients based on increased IL-17 expression in muscle tissue(25). Multiple biologic drugs targeting the IL-17 and IL-6 pathway are in clinical use(27, 28). The heterogeneity in IL-17 and IL-6 expression reported here implies that not all IIM patients would be responsive to such inhibition.

This study confirms the existence of an expanded Th17 population in the peripheral blood of IIM patients (29, 30). Based on the results presented here, it would be important to establish a correlation between the proportion of Th17 cells in the

periphery and inflamed tissue at diagnosis and to monitor the change over time on treatment with disease progression.

Previously AM patient subtypes have been described based on autoantibody profiles (31), which was also reflected in this analysis. Myositis associated antibodies are detected in both DM and PM, but there is a wider range found in DM. The anti-Jo1 and anti-SRP autoantibodies are more common in PM. However, anti-Mi2, anti-TIF1 γ , and anti-NXP2 are rarely detected in PM but more commonly in DM and a-JDM (32). The AM cohort for this study displayed comparable prevalence of myositis associated antibodies to that previously reported. In particular, PM patients were only positive for anti-Jo1 and anti-SRP. Unfortunately, accurate data was not available for the a-JDM cohort.

B cells have been detected within the inflamed muscle of AM and JDM patients, suggesting that B cells exert a pathological function on muscles (12, 33). A notable autoantibody profile can be detected in most IIM patients implying one function of B cells in this group of diseases is the production of autoantibodies (34). These results provided an insight into the phenotype and function of B cells within the peripheral blood of both AM and a-JDM patients. The B cell compartment was dysregulated in the peripheral blood; the AM patients had a lower proportion of CD27⁻ and BM5 early (IgD-CD38⁺) B cells compared to a-JDM and AHC groups, and the a-JDM group had an increased BM2 transitional (IgD^{hi}CD38^{hi}) population. However, studies in SLE have identified that the memory B cell populations, both CD27⁺ and ⁻, were expanded and immunosuppressive resistant (35). The decrease of the CD27⁺ and BM5 B cell populations was most evident in the AM patients expressing anti-synthetase (anti-Jo-1 and anti-Pl-12) autoantibodies and those that were classified as in remission off treatment. Interestingly Piper et al have previously detailed an in-depth investigation of B cell populations in JDM from pre- and on-treatment patients, they showed that the memory B cell population was lowest in pre-treatment patients (16). The low memory B cell population identified in IIM may be a coincidental non-pathogenic finding which better reflects changes in related populations. Interestingly, there was an increased Plasmablast (BM3-4) population seen in the ADM patients

with cancer with associated anti-TIF1 γ and anti-NXP2 positivity. This maybe a signature associated with the underlying cancer or specific antibody production(36).

The limitations of this study were that we did not have access to treatment naïve patient samples, and therefore it was not possible to delineate the effect of treatment on the disease course and immune signatures. However, it is difficult to recruit adult patients at diagnosis naïve of treatment as the disease is very rare and patients frequently experience delayed or wrong diagnosis with previous steroid use(37). It would be important for future studies to define any changes in the immune signature over the treatment course, and therefore would require longitudinal samples that were matched disease activity scores.

In conclusion, this study has identified IL-17 and IL-6 as potential therapeutic targets in ADM patients but also revealed complex patterns of immune cell expression in APM and a-JDM patients that provide a base for future investigations focused on understanding the disease pathogenesis and development of more personalised choice of biologic therapies for patients with IIMs.

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Table 1:

	Patients						Controls		
Patient characteristics	Adult Myositis samples (n=44)						Juvenile DM (adolescent onset) samples (n=15)	Adult healthy control samples (n=25)	Adolescent healthy control samples (n=15)
Sub-types of myositis	All	Dermatomyositis	Polymyositis	Dermatomyositis with cancer	Dermatomyositis with overlap syndrome	Polymyositis with overlap syndrome	All	All	All
Number of patients	44	19	9	4	7	5	15	25	15
Age at diagnosis (years), median [IQR] (p=0.0632)	39.82 [31.65-49.00]	34.02 [30.33-48.96]	41.29 [34.10-44.07]	47.83 [27.65-60.62]	39.87 [31.30-45.80]	39.64 [32.47-43.04]	14.78 [14.02-16.01]		
Age at sample (years), median [IQR] (p=0.2194)	55.48 [47.59-60.33]	55.80 [46.21-59.99]	52.37 [48.35-53.10]	63.04 [59.81-67.99]	49.20 [46.82-53.47]	59.72 [55.96-66.73]	21.48[19.07-23.19]	51.26[43.23-58.31]	20.11[17.40-22.28]
Time since diagnosis (years), median [IQR] (p=0.5453)	9.64 [3.39-23.13]	12.39 [4.61-22.29]	6.43 [5.15-10.97]	0.34 [0-6.23]	11.02 [1.02-24.86]	23.48 [7.98-23.69]	6.09 [5.28-7.82]		
Sex, (F/M) [p=0.3013]	34/10	12/7	9/0	4/0	5/2	4/1	11/4	14/11	11/4
Ethnicity, n (%) [0.0968]									
White British	17 (38.64)	10 (52.63)	1 (11.11)	2 (50.00)	3 (42.86)	1 (20)	9 (60.00)	14 (56.00)	6 (40.00)
Black Caribbean	9 (20.45)	1 (5.26)	5 (55.56)	1 (25)	1 (14.29)	1 (20)	2 (13.33)	0 (0.00)	0 (0.00)
Black African	3 (6.81)	1 (5.26)	0 (0.00)	0 (0.00)	1 (14.29)	1 (20)	1 (6.67)	0 (0.00)	1 (6.67)
South Asian	7 (15.91)	2 (10.53)	1 (11.11)	0 (0.00)	2 (28.57)	2 (40.00)	0 (0.00)	2 (8.00)	5 (33.33)
East Asian	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (6.67)
White other	7 (15.91)	4 (21.05)	2 (22.22)	1 (25)	0 (0.00)	0 (0.00)	0 (0.00)	7 (28.00)	0 (0.00)
South American	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Other	1 (2.27)	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (20.00)	0 (0.00)	1 (6.67)
Unknown	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (8.00)	0 (0.00)

Table 2:

Patient characteristics	Adult Myositis samples (n=44)						Juvenile DM (adolescent onset) samples (n=15)
Sub-types of myositis	All	Dermatomyositis (n=19)	Polymyositis (n=9)	Dermatomyositis with cancer (n=4)	Dermatomyositis with overlap syndrome (n=7)	Polymyositis with overlap syndrome (n=5)	All
ESR mm/hour [p=0.0394]	18.5 [8.00-28.25]	9.00 [5.75-17.50]	25.00 [22.5-30.00]	40.00 [33.00-43.50]	23.00 [16.50-37.50]	24.5 [16.25-36.25]	16.5 [7.50-22.50]
CRP mg/L [p=0.7019]	2 [0.70-5.80]	1.60 [0.78-2.65]	2.3 [0.70-6.48]	3.00 [1.88-28.45]	4.45 [1.73-17.90]	4.90 [1.65-10.05]	2.75 [2.23-5.00]
CK U/L [p=0.6966]	147 [93-364]	195 [125-301]	230.5 [142-308]	116 [107.75-696.50]	100 [92.25-949.25]	87 [75.5-192.25]	106 [76.50-464.75]
MITAX score (p=0.063)	1.50 [0.00-9.00]	1.00 [0.00-9.00]	3.00 [1.00-9.00]	9.50 [1.75-19.00]	2.00 [0.50-10.50]	0.00 [0.00-2.25]	0.00 [0.00-4.00]
MITAX skin score	0 [0-12]						0 [0-8]
MMT8 (0-80)	Not done	Not done	Not done	Not done	Not done	Not done	80 [77-80]
Myositis-specific autoantibodies, n (%) [p=0.3022]							
Anti-Jo-1	6 (13.63)	1 (5.26)	4(44.44)	0 (0.00)	1(14.29)	0 (0.00)	0 (0.00)
Anti-Ro	3 (6.81)	2 (10.53)	0 (0.00)	1 (25)	0 (0.00)	0 (0.00)	1 (13.33)
Anti-PL12	1 (2.27)	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Anti-PM-Scl75	2 (4.54)	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)	1 (20)	0 (0.00)
Anti-Mi2	3 (6.81)	3 (15.78)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (6.67)
Anti-Smith	1 (2.27)	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Anti-SRP	1 (2.27)	0 (0.00)	1 (11.11)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Anti-NXP2	1 (2.27)	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
TIF1-gamma	3 (6.81)	2 (10.53)	0 (0.00)	1 (25)	0 (0.00)	0 (0.00)	0 (0.00)
Multiple	9 (20.45)	2 (10.53)	0 (0.00)	0 (0.00)	4 (57.14)	3 (60)	5 (33.33)
Negative	7 (15.90)	3 (15.78)	3 (33.33)	1 (25)	0 (0.00)	0 (0.00)	0 (0.00)
Not done	7 (15.90)	2 (10.53)	1 (11.11)	1 (25)	2 (28.57)	1 (20)	3 (20.00)
Medications (at time of sample) n(%) [p=0.3237]							
Prednisolone	28 (63.63)	10 (52.63)	4 (44.44)	3 (75)	7 (100)	4 (80)	8 (53.33)
Methotrexate	5 (11.36)	2 (10.53)	2 (22.22)	0 (0.00)	1 (14.29)	0 (0.00)	10 (66.67)
Azathioprine	9 (20.45)	2 (10.53)	2 (22.22)	1(25.00)	4 (57.14)	0 (0.00)	2 (13.33)
Rituximab past	7 (15.91)	2 (10.53)	2 (22.22)	0 (0.00)	1 (14.29)	2 (40.00)	1 (6.67)
Hydroxychloriquine	5 (11.36)	3 (15.78)	1 (11.11)	0 (0.00)	1 (14.29)	0 (0.00)	6 (40.00)
MMF	8 (18.18)	5 (26.32)	1 (11.11)	0 (0.00)	1 (14.29)	1 (20)	2 (13.33)
Tacrolimus	3 (6.812)	1 (5.26)	1 (11.11)	0 (0.00)	1 (14.29)	0 (0.00)	0 (0.00)
Cyclosporine	2 (4.54)	2 (10.53)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
IVIg	3 (6.82)	2 (10.53)	0 (0.00)	1 (25)	0 (0.00)	0 (0.00)	0 (0.00)
Infliximab	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (13.33)

Adalimumab	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (6.67)
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FIGURE LEGENDS

Table 1: Demographic features of the adult myositis, adolescent-onset-JDM, adult and teenage healthy control cohorts at time of sample. 44 adult myositis (AM) patients and 15 adolescent onset-juvenile dermatomyositis (a-JDM) patients (adolescence defined as 10-19 years of age) were recruited. The AM group was divided into subgroups; dermatomyositis (ADM), polymyositis (APM), dermatomyositis with cancer, dermatomyositis with overlap and polymyositis with overlap.

Table 2: Clinical and serological features of the adult myositis, adolescent-onset-JDM, adult and teenage healthy control cohorts at time of sample. 44 adult myositis (AM) patients and 15 adolescent onset-(a)-juvenile dermatomyositis (a-JDM) patients (adolescence defined as 10-19 years of age) were recruited. The AM group was divided into subgroups; dermatomyositis (ADM), polymyositis (APM), dermatomyositis with cancer, dermatomyositis with overlap and polymyositis with overlap. For each group, the clinical and serological data were recorded at time of PBMC sample including; erythrocyte sedimentation rate (ESR) [normal range <20mm/hour], C-reactive protein (CRP) [normal range <5mg/L], creatine kinase (CK) [normal range <150U/L], MITAX score, MITAX skin score (median with range) MMT8 score [normal range >78], auto-antibodies and medication. The binary measures were analysed by Chi-square test and the continuous variables were analysed by one-way ANOVA. $p \leq 0.05$ for significance.

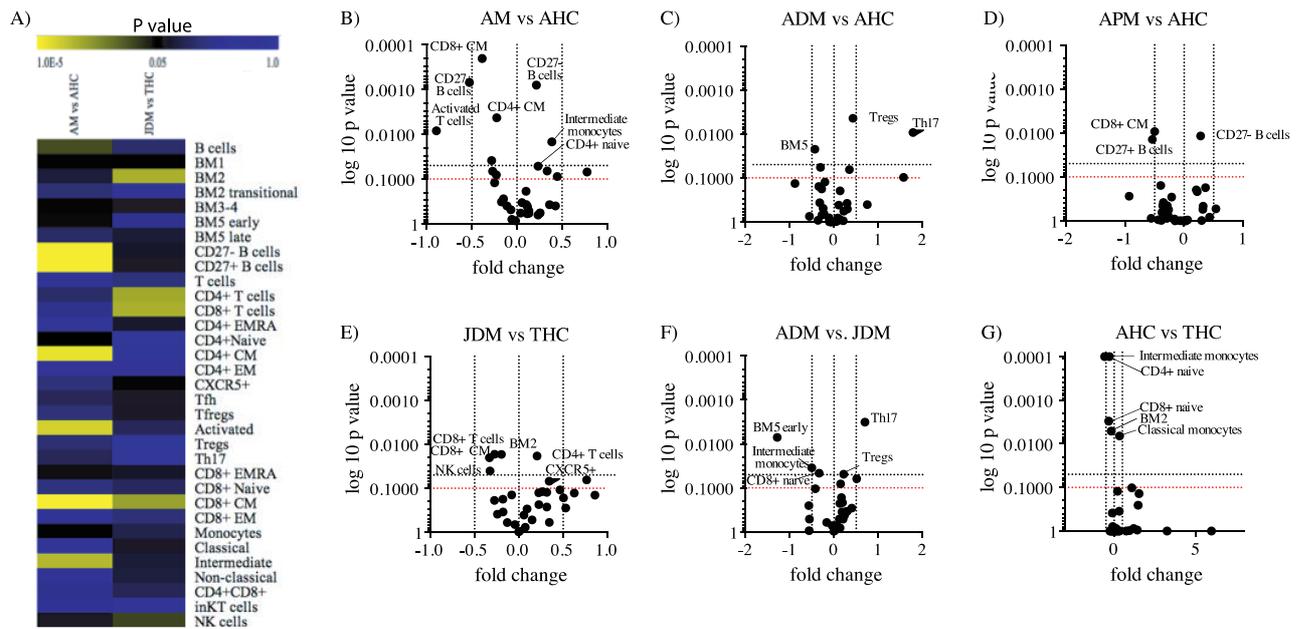


Figure 1: Heterogeneous immune cell profiles in IIM patient subgroups. The frequency of 33 PBMC sub-populations were assessed by multiparameter flow cytometry from 44 adult myositis (AM) patients, 25 adult healthy controls (AHC), 15 adolescent-onset juvenile dermatomyositis (a-JDM) patients and 15 teenage healthy controls (THC). **(A)** Heat map demonstrating the statistical difference between each cell population by comparing the mean of each group; AM vs. AHC and a-JDM vs. THC. A student t-test calculated the p-values. Black to yellow – $p < 0.05$. Blue to black – $p > 0.05$. Volcano plots are 2-D scatter plots that visualise significance, direction and the magnitude of changes within a given comparison. Volcano plots showing log₂ scale fold change (x-axis) and log₁₀ scale p value (y-value) for each cell population when sample groups were compared. A negative fold change indicates that the cell population for the first named patient group was lower than the second named patient or healthy control group. A positive fold change indicates that the cell population for the first named patient group was higher than the second named patient or healthy control group. **(B)** AM) vs. AHC), **(C)** adult dermatomyositis (ADM) (n=19) vs. AHC), **(D)** adult polymyositis (APM) (n=9) vs. AHC, **(E)** a-JDM vs. THC, **(F)** ADM vs. a-JDM **(G)** AHC vs. THC. P values were calculated by one-way ANOVA. All p-values represent adjusted p-values calculated by Tukey’s multiple comparison. Stringent significance $p \leq 0.05$ (horizontal black dotted line). Adjusted significance by false discovery rate (FDR) for small sample size $p \leq 0.1$ (horizontal red dotted line). The fold change significance is ≤ -0.5 and ≥ 0.5 (vertical dotted black lines).

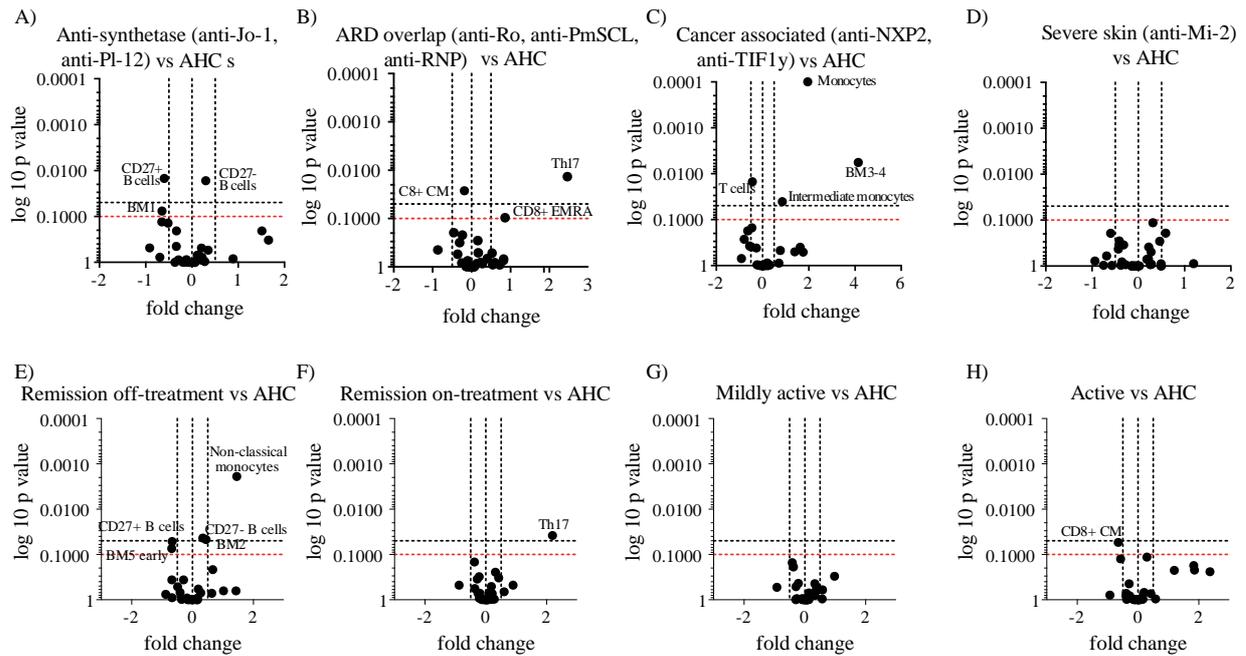


Figure 2: Heterogeneous immune cell profiles in IIM patient subgroups: autoantibody and disease activity. Volcano plots showing fold change (x-axis) and p value (Y-axis) for each cell population when AM autoantibody groups were compared with AHC samples. **A)** Anti-synthetase group (anti-Jo-1 and anti-Pl-12) (n=7) compared to AHC (n=25). **B)** Autoimmune rheumatic disease overlap (ARD) group (anti-Ro, anti-PmScl and anti-RNP) (n=9) compared to AHC. **C)** Cancer associated group (anti-NXP2 and anti-TIF1 γ) (n=4) compared to AHC. **D)** Severe skin group (anti-Mi2) (n=3) compared to AHC. Volcano plots represent the fold change and p value for each cell population when AM disease activity groups were compared. Patients' with cancer, pregnant, treated with rituximab or diagnosed in childhood were removed from this analysis. **E):** Remission off-treatment (n=5) compared to AHC (n=25). **F)** Remission on-treatment (n=12) compared to AHC (n=25). **G)** Mildly active (n=9) compared to AHC (n=25). **H)** Active (n=4) compared to AHC (n=25). P values were calculated by one-way ANOVA. All p-values represent adjusted p-values calculated by Tukey's multiple comparison. Stringent significance $p \leq 0.05$ (horizontal black dotted line). Adjusted significance by false discovery rate (FDR) for small sample size $p \leq 0.1$ (horizontal red dotted line). The fold change significance is ≤ -0.5 and ≥ 0.5 (vertical dotted black lines).

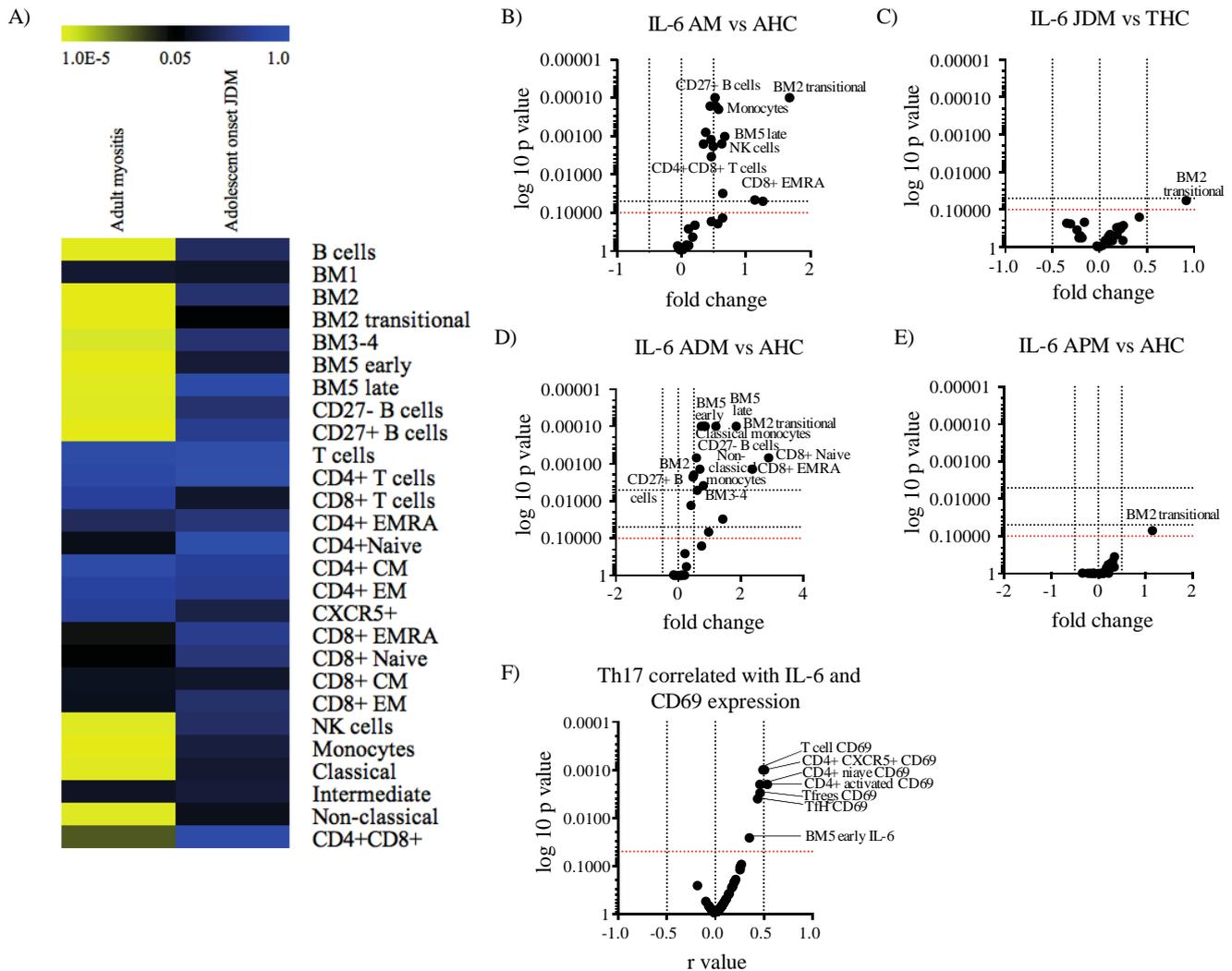


Figure 3: Significant differences in cell populations expression of IL-6 and the Th17 population, a comparison of IIM to healthy samples. IL-6 expression in 27 PBMC subpopulations was assessed by flow cytometry from 44 adult myositis (AM) patients, 25 adult healthy controls (AHC), 15 adolescent-onset juvenile dermatomyositis (a-JDM) patients and 15 teenage healthy controls (THC). **A)** Heat map showing the statistical difference between each cell population by comparing the mean of each group; AM vs. AHC, and a-JDM vs. THC. A student t-test calculated the p-values. Black to yellow - $p \leq 0.05$. Blue to black - $p \geq 0.05$. Volcano plots identifying significant differences and fold change of IL-6 expression in immune cell populations. **B)** AM (n=44) compared to AHC (n=25). **C)** a-JDM (n=15) compared to THC (n=15). **D)** ADM (n=19) compared to AHC (n=25). **E)** APM (n=9) compared to AHC (n=25). P values were calculated by multiple t-test, significance $p \leq 0.05$ (bottom horizontal dotted black line). Multiple comparison was calculated by the Holm-Sidak method, therefore adjusted p value significance $p \leq 0.005116$ (top

horizontal dotted black line). Adjusted significance by FDR for small sample size $p \leq 0.1$ (red dotted line). The fold change significance is ≤ -0.5 and ≥ 0.5 (vertical dotted black lines). **F)** A volcano plot showing all PBMC populations, from the total AM group, expression of CD69 and IL-6 correlation to Th17 frequency. Pearson's correlation was conducted, positive r value represents a positive correlation and negative r value represents a negative correlation. Significance reached $p \leq 0.05$ (horizontal dotted red line).

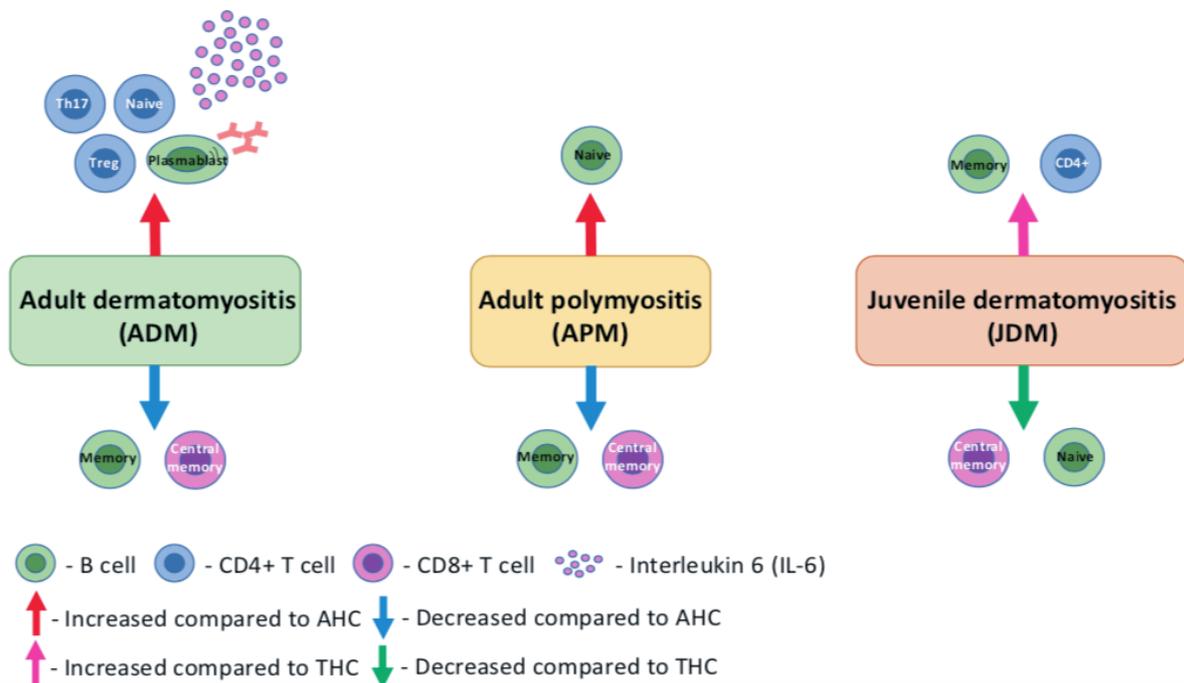


Figure 4: Different immune signatures in adult dermatomyositis, polymyositis and juvenile dermatomyositis. Different immune signatures in adult dermatomyositis and polymyositis compared to adult healthy control and adolescent-onset juvenile dermatomyositis compared to teenage healthy control. ADM was characterised by an increase of CD4⁺ T-cells including; Th17, naïve T-cells and Tregs, but also plasmablasts in cancer associated dermatomyositis and an increased cellular expression of IL-6. Common to both ADM and APM there was a decrease of memory B-cells and central memory CD8⁺ T-cells. This decrease in central memory CD8⁺ T-cells was also noted in a-JDM.