Clin Rheumatol (2012) 31:1691–1697
DOI 10.1007/s10067-012-2075-4

ORIGINAL ARTICLE

Clinical significance of peripheral blood lymphocyte subsets in patients with polymyositis and dermatomyositis

Dong Xue Wang · Xin Lu · Ning Zu · Bing Lin · Li Ying Wang · Xiao Ming Shu · Li Ma · Guo Chun Wang

Received: 5 June 2012 / Revised: 3 August 2012 / Accepted: 19 August 2012 / Published online: 30 August 2012

Abstract Peripheral blood lymphocyte subsets were determined by flow cytometry in 89 Chinese patients with polymyositis (PM) and dermatomyositis (DM). We aimed to investigate the clinical significance of peripheral blood lymphocyte subsets in PM/DM. Patients with active DM showed significant decreases in numbers of $CD3^+$ cells, $CD3^+CD4^+$ cells, and $CD3^+CD8^+$ cells, as compared to patients with inactive DM and healthy controls ($P<0.05$). $CD3^+$ and $CD3^+CD4^+$ cell counts were significantly lower in DM before treatment, compared with after treatment ($t=-5.714$ and -3.665, $P<0.05$). Counts of $CD3^+$ cells, $CD3^+CD4^+$ cells, $CD3^+CD8^+$ cells, and $CD19^+CD5^-$ cells were all correlated with the total disease activity score as determined by the Myositis Disease Activity Assessment Visual Analogue Scale ($P<0.05$). The decreased number of $CD3^+$ cells and the decreased percentage of $CD3^+CD4^+$ cells were additionally correlated with the presence of interstitial lung disease in PM/DM ($P<0.05$). The presence of levels of $CD3^+CD8^+$ cells was risk factor for death ($b=-0.011$, OR=0.989, $P<0.05$). The identification of peripheral blood T lymphocyte subsets in PM/DM appears to be useful as a reference marker in the evaluation of clinical disease activity, and be useful in the comprehensive assessment of clinical lung involvement. A decrease in $CD8^+$ T cells may predict a poor outcome in patients with PM/DM.

Keywords Dermatomyositis · Flow cytometry · Lymphocyte subsets · Polymyositis

D. X. Wang · X. Lu · N. Zu · B. Lin · L. Y. Wang · X. M. Shu · L. Ma · G. C. Wang (✉)
Department of rheumatology, China-Japan Friendship hospital, Yinghua East road, Chaoyang district, Beijing 100029, China
e-mail: guochunwang@hotmail.com

Introduction

The idiopathic inflammatory myopathies (IIMs) are systemic autoimmune diseases characterized by chronic inflammation of skeletal muscle. Polymyositis (PM) and dermatomyositis (DM) are two common subtypes of IIM. The diagnosis is based on a combination of clinical examination, serum muscle enzyme levels, electromyographic data, and muscle biopsy findings. Although the cause of PM/DM is incompletely understood, an autoimmune mechanism is thought to be central to the underlying pathogenesis. Previous studies have revealed that PM is characterized by $CD8^+$ T-cell-mediated cytotoxicity against the major histocompatibility complex class I expressed by muscle fibers. This is in contrast to DM, in which $CD4^+$ T cells and B cells are known to predominate in the perivascular areas of the muscle tissue, and complement-mediated injury directed against the intramuscular microangiopathy is thought to be more significant [1].

In order to gain better insight into the pathogenesis of PM/DM, investigation of lymphocyte subsets and its functions may be important. However, there are few studies available that have examined the clinical significance of peripheral blood lymphocyte subsets in patients with PM/DM, and the reported results have been controversial thus far [2, 3]. The aim of this study was to determine the levels of peripheral blood lymphocyte subsets by flow cytometry in a relatively larger patient population with PM/DM, and to subsequently investigate their association with clinical features.

Materials and methods

Patients and controls

The study was conducted during 2007–2011 and it was approved by the Research Ethics Committee of China-

Japan Friendship Hospital, the Ministry of Health. All patients and controls gave written informed consent. In total, 89 patients with a diagnosis of PM/DM according to the Bohan & Peter criteria [4, 5] who were treated at the outpatient and inpatient departments of rheumatology at China-Japan Friendship Hospital were recruited into this study. Seventeen patients were newly diagnosed and previously untreated. The others had already been treated with corticosteroid and/or immunosuppressive agent as an initial therapy or a maintenance therapy. Sixty healthy volunteers were selected as the control group during the same time period.

Clinical activity of muscle and other organ involvement was determined using the Myositis Disease Activity Assessment Visual Analogue Scale (MYOACT) recommended by the International Myositis Outcome Assessment Collaboration Study [6], and was recorded as a MYOACT-muscle disease score, MYOACT-cutaneous disease score, and MYOACT-total disease activity score. Clinical data was coded at time of enrollment and included gender, age, disease course, MYOACT-muscle disease score, MYOACT-cutaneous disease score, MYOACT-total disease activity score, serum creatine kinase (CK), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), disease type (PM or DM), interstitial lung disease (ILD), malignancy, infection, antinuclear antibody (ANA) positivity, anti-Jo-1 antibody positivity, any treatment before enrollment, corticosteroid treatment before enrollment, immunosuppressive drug therapy (including methotrexate, cyclophosphamide, hydroxychloroquine, etc.) before enrollment. age, disease course, MYOACT scores, CK, LDH, ESR, and CRP were recorded as quantitative data, whereas gender was recorded as 0 (male) or 1 (female), disease type was recorded as 0 (PM) or 1 (DM). ILD, malignancy, infection, ANA positivity, anti-Jo-1 antibody positivity, and treatments were also recorded as 0 or 1, representing the absence or presence of the corresponding clinical features. Patients were considered to be in complete disease remission if they maintained a total score of 0 points for at least 1 month based on the MYOACT-muscle and MYOACT-cutaneous disease scores and the MYOACT-total disease activity score. Those patients who did not satisfy the criteria above were considered to have active disease.

Demographic details and clinical features of the patients at the time of enrollment are shown in Table 1. All patients with PM/DM were followed up for 1 month to 40 months, and the disease conditions were monitored. Among those 89 patients, six newly diagnosed patients with DM were followed up for 0.5 to 12 months to detect the peripheral blood lymphocyte subsets for the second time.

Measurement of peripheral blood lymphocyte subsets

A 4-ml EDTA-k2 anticoagulant blood sample was taken from all patients and controls at the enrollment. Of this sample, 2 ml was used for measuring whole blood lymphocyte counts by Sysmex SF-3000 automated hematology analyzer (Sysmex Zhushikuaishe, Japan). Then samples of 100 μl were added into three separate tubes, incubated with monoclonal antibodies CD4-FITC/CD8-PE/CD3-PC5 in tube 1, CD3-FITC/CD16CD56-PE in tube 2, and CD5-FITC/CD19-PE in tube 3 (Immunotech, France), for 30 min at room temperature in the dark. Whole blood samples were then lysed according to the protocol provided by the Immuno-prepTM hemolytic process kit (Beckman Coulter, USA). The same technique was applied for the control antibody (IgG1-FITC, IgG1-PE, and IgG1-PC5, Immunotech, France). The samples were measured by Epics-XL flow cytometer (Beckman Coulter, USA). The gate was set on lymphocytes, and 1×10^4 cells were analyzed to determined percentages of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD16^+CD56^+$, and $CD19^+CD5^-$ phenotypes. Then absolute counts of $CD3^+$ cells, $CD3^+CD4^+$ cells, $CD3^+CD8^+$ cells, $CD3^-CD16^+CD56^+$ cells, and $CD19^+CD5^-$ cells were also determined by multiplying the percentage of lymphocyte subsets with whole blood lymphocyte counts.

Table 1 Demographic and clinical features of PM/DM patients

	PM (n=19)	DM (n=70)	HC (n=60)
Male/female (n)	4/15	17/53	21/39*
Newly diagnosed (n)	2	15	
Mean age (years)	48.00±14.21	50.96±14.01	47.63±8.45*
Disease course (months)	18.79±28.25	16.89±20.75	
CK (IU/L)	1,587.11±2,233.79	289.07±591.96	
LDH (IU/L)	460.37±272.79	317.59±150.89	
CRP (mg/dl)	1.73±3.14	1.18±2.08	
ESR (mm/h)	36.27±26.85	44.16±29.29	
Anti-Jo-1 positivity (n)	2	3	
ANA positivity (n)	13	25	
With ILD (n)	8	36	

PM polymyositis, *DM* dermatomyositis, *HC* healthy controls, *CK* creatine kinase, *LDH* lactate dehydrogenase, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *ANA* antinuclear antibody, *ILD* interstitial lung disease

*P>0.05 (HC vs. PM or DM)

Statistical analysis

The SPSS15.0 statistical analysis software was used for data processing. ANOVA was employed to determine the statistical significance across different groups. Independent sample t tests and χ^2 tests were used to compare each variable between groups for univariate analysis, following a backward method of internalizing variables into the multivariate logistic regression analysis. The statistical difference was considered significant if $P<0.05$.

Results

Peripheral blood lymphocyte subsets in active and inactive DM

The DM group consisted of 15 previously untreated patients with active disease (aDM), and ten previously treated patients with inactive disease (iDM). In the PM group, there were only two newly diagnosed patients, so comparisons in this group were not made. Lymphocyte subsets of the aDM, iDM, and the healthy control group (HC) are shown in Table 2. Counts of $CD3^+$ cells, $CD3^+CD4^+$ cells, and $CD3^+CD8^+$ cells in the aDM group were all significantly lower than in the HC and iDM groups ($P<0.05$). Counts of $CD3^+$ cells, $CD3^+CD4^+$ cells, and $CD3^+CD8^+$ cells nearly increased to normal levels in iDM individuals, and the differences were not statistically significant when compared to the HC group ($P>0.05$). Percentages of $CD19^+CD5^-$ cells in both the aDM and iDM groups were significantly higher than those in the HC group ($P<0.05$). $CD3^-CD16^+CD56^+$ cells as well as counts of $CD3^-CD16^+CD56^+$ cells in both the aDM and iDM groups were significantly lower than those in the HC group ($P<0.05$).

Peripheral blood lymphocyte subsets in DM before and after clinical remission

Six newly diagnosed patients were followed up to detect peripheral blood lymphocyte subsets. Each patient received clinical remission after treatment. All of the six patients were treated with corticosteroid, and only one patient was treated with immunosuppressive agent at the same time. Their $CD3^+$ and $CD3^+CD4^+$ cell counts were 514.0±412.1 and 255.0±127.0 cell/mm^3 at the enrollment, which were significantly lower compared with the values, 833.5± 470.5 and 449.2±146.0 cell/mm^3, respectively, after therapy ($t=-5.714$ and -3.665, $P<0.05$). The values of the other peripheral blood lymphocyte subsets after therapy were not significantly different from the initial values before therapy ($P>0.05$). Results are demonstrated in Table 3 and Fig. 1.

Table 2 Peripheral blood lymphocyte subsets in patients with active and inactive DM

Group	$CD3^+$ cell/mm^3	$CD3^+CD4^+$ cell/mm^3	$CD3^+CD8^+$ cell/mm^3	$CD19^+CD5^-$ cell/mm^3	$CD3^-CD16^+CD56^+$ cell/mm^3
HC	1,376.3±389.7	834.4±278.6	457.9±170.4	138.8±63.1	288.9±113.7
aDM	848.7±446.6*	534.8±279.8*	257.2±157.9*	185.4±153.6	192.1±276.9*
iDM	1,632.4±622.5**	1,041.8±558.2**	562.5±376.2**	312.4±186.4**	153.2±169.9*
Group	$CD3^+$ (%)	$CD3^+CD4^+$ (%)	$CD3^+CD8^+$ (%)	$CD19^+CD5^-$ (%)	$CD3^-CD16^+CD56^+$ (%)
HC	70.4±8.4	42.5±8.1	23.6±6.7	6.9±2.1	14.9±5.9
aDM	65.5±9.9	42.8±10.3	20.7±4.7	13.9±8.3*	10.2±9.5*
iDM	73.9±8.7**	48.3±17.3	23.9±14.5	14.2±7.0*	7.7±6.3*

HC healthy controls, *aDM* active dermatomyositis, *iDM* inactive dermatomyositis

*$P<0.05$ (HC vs. aDM or iDM); **$P<0.05$ (aDM vs. iDM)

The association of clinical features with peripheral blood lymphocyte subsets

The normal reference range of every lymphocyte subset was determined to be within the 95 % interval of lymphocyte subsets in the HC group. According to the normal reference ranges, patients with PM/DM were divided into different groups based on the levels of lymphocyte subsets. The relationship between each lymphocyte subset group and associated clinical features was evaluated separately using multivariate logistic regression analysis as listed in Table 4. Initially, only eight variables (gender, MYOACT-cutaneous disease score, MYOACT-total disease activity score, LDH, disease type, ILD, anti-Jo-1 antibody positivity, and immunosuppressive drug therapy) were identified as significant factors by independent sample t tests and χ^2 tests. Then independent clinical variables affecting peripheral blood lymphocyte subsets were analyzed by including the above variables into the logistic regression model. MYOACT-total disease activity scores were positively correlated with decreased counts of $CD3^+$ cells, $CD3^+CD4^+$ cells, and $CD3^+CD8^+$ cells, and negatively correlated with high $CD19^+CD5^-$ cell count ($P<0.05$). The use of immunosuppressive drug therapy was positively correlated with decreased counts of $CD3^+CD4^+$ cells and $CD3^+CD8^+$ cells ($P<0.05$). Low levels of $CD3^+$ cells and low percentages of $CD3^+CD4^+$ cells were positively correlated with the presence of ILD in PM/DM ($P<0.05$). Multivariate analysis also showed that low percentages of $CD3^+CD4^+$ cells and disease type (PM or DM) were related, where reduced $CD3^+CD4^+$ cells were more likely to be observed in patients with DM ($P<0.05$).

Association with peripheral blood lymphocyte subsets and disease prognosis

There were four patients lost to follow-up, yielding a response rate of 95.5 % (85/89). In addition, seven patients died either during the hospital course or during the follow-up period. Of these, 6/7 (85.7 %) had peripheral blood lymphocyte subsets that were abnormal at the time of enrollment. Lymphocyte subset distribution of those patients is listed in Table 5. Their counts of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ cells were significantly decreased at the time of enrollment. Univariate analysis was performed between living subset and dead subset and significant factors suspected to be related with death were chosen and used in multivariate analysis. Finally, six risk factors suspected to be related with death were identified, which were disease course, MYOACT-total disease activity score, ANA positivity, the presence of malignancy, counts of $CD3^+$ cells, and counts of $CD3^+CD8^+$ cells at enrollment. Logistic regression analysis indicated that counts of $CD3^+CD8^+$ cells and the presence of a comorbid malignant tumor were independent risk factors for death ($P<0.05$), yielding relative risks of 0.989 and 131.9, respectively (Table 6).

Discussion

Lymphocyte-mediated immune mechanisms are thought to play an important role in the pathogenesis of PM/DM. Many studies have previously confirmed that muscle tissue of patients with PM/DM is characterized by lymphocytic infiltration, whereas studies also indicate that almost no lymphocytes are localized in normal muscle tissues [1, 7]. After transferring lymphocytes from autoimmune myositis model rats to a group of healthy rats, Kojima et al. observed the development of mild clinical features in the group of healthy animals. These clinical features were similar to the findings found in the model animals [8]. Ishii et al. reported that patients with PM/DM showed significant increases in peripheral blood $CD19^+CD23^+$ cells ($p<0.05$), and significant decreases in $CD4^+CD45RO^+$ cells ($p<0.05$) as compared with controls [2]. In our study, we used a logistic regression model to investigate the relationship

Table 3 Peripheral blood lymphocyte subsets in DM before and after clinical remission

	Before treatment	After treatment
CK	888.0±957.7	291.2±5.7.0
MYOACT- total disease activity score	6.2±1.0	3.0±2.5*
$CD3^+$ (cell/mm^3)	514.0±412.1	833.5±470.5*
$CD3^+CD4^+$ (cell/mm^3)	255.0±127.0	449.2±146.0*
$CD3^+CD8^+$ (cell/mm^3)	229.8±289.3	357.3±412.9
$CD19^+CD5^-$ (cell/mm^3)	131.8±138.4	194.6±75.9
$CD3^-CD16^+CD56^+$ (cell/mm^3)	137.7±205.5	150.5±254.5
$CD3^+$ (%)	62.7±7.6	68.1±7.3
$CD3^+CD4^+$ (%)	34.8±10.5	41.6±17.6
$CD3^+CD8^+$ (%)	22.9±8.2	24.2±12.9
$CD19^+CD5^-$ (%)	13.7±4.2	16.7±5.0
$CD3^-CD16^+CD56^+$ (%)	10.6±5.7	6.6±8.5

*$P<0.05$ (before treatment vs. after treatment)

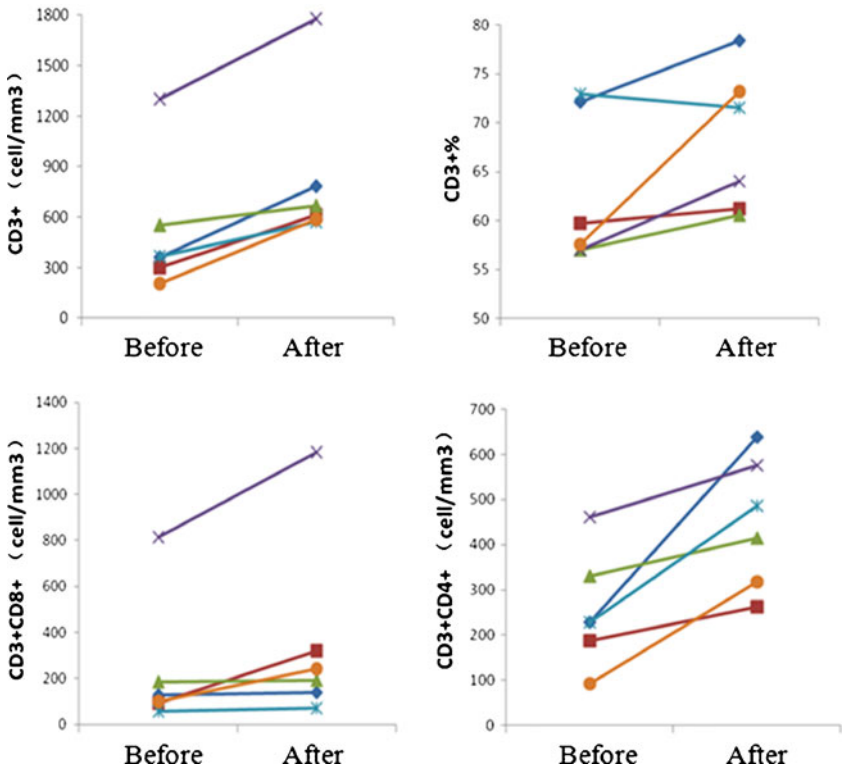

Fig. 1 Lymphocyte subsets in DM before and after treatment

between peripheral blood lymphocyte subsets and disease characteristics of patients with PM/DM. We found that gender, age, disease duration, ANA positivity, anti-Jo-1 antibody positivity, infection, malignancy, corticosteroid treatment etc. do not independently exhibit a clinically significant effect on the level of peripheral blood lymphocyte subsets, while the

Table 4 The association of clinical features with peripheral blood lymphocyte subsets

	Independent variables	b[a]	S.E.[a]	χ_{Wald}^2 [a]	P value[a]
Low $CD3^+$ cells count	MYOACT-total disease activity score	0.211	0.090	5.540	0.019
	ILD	0.928	0.457	4.130	0.042
Low $CD3^+CD4^+$ cells count	MYOACT-total disease activity score	0.344	0.116	8.818	0.003
	Immunosuppressive drugs	1.736	0.678	6.548	0.010
Low $CD3^+CD8^+$ cells count	MYOACT-total disease activity score	0.289	0.109	6.981	0.008
	Immunosuppressive drugs	1.303	0.616	4.471	0.034
High $CD19^+CD5^-$ cells count	MYOACT-total disease activity score	−0.261	0.118	4.944	0.026
	LDH	0.004	0.002	5.985	0.014
Low $CD3^-CD16^+CD56^+$ cells count	NA	NA	NA	NA	NA
Low $CD3^+$ cells percentage	NA	NA	NA	NA	NA
Low $CD3^+CD4^+$ cells percentage	Disease type (PM or DM)	1.666	0.740	5.067	0.024
	ILD	1.974	0.859	5.278	0.022
Low $CD3^+CD8^+$ cells percentage	NA	NA	NA	NA	NA
High $CD19^+CD5^-$ cells percentage	NA	NA	NA	NA	NA
Low $CD3^-CD16^+CD56^+$ cells percentage	NA	NA	NA	NA	NA

Normal reference ranges of percentage (%) of lymphocyte subsets: $CD3^+$ cells (50.7~81.9), $CD3^+CD4^+$ cells (12.5~34.8), $CD3^+CD8^+$ cells (23.4~47.5), $CD19^+CD5^-$ cells (2.8~9.5), and $CD3^-CD16^+CD56^+$ cells (5.9~26.4); normal reference ranges of count (cell/mm^3) of lymphocyte subsets: $CD3^+$ cells (818~2,007), $CD3^+CD4^+$ cells (383~1,385), $CD3^+CD8^+$ cells (224~729), $CD19^+CD5^-$ cells (53~267), and $CD3^-CD16^+CD56^+$ cells (170~529)

PM polymyositis, *DM* dermatomyositis, *LDH* lactate dehydrogenase, *ILD* interstitial lung disease, *NA* no independent clinical variables affecting that lymphocyte subset, *b* regression coefficient, *S.E.* standard error

[a] Statistical results obtained using logistic regression

Table 5 Features of peripheral blood lymphocyte subsets in dead patients

	Gender	Age (year)	Disease course (month)	ANA	Lymphocyte subsets (cell/mm^3)					Cause of death
					$CD3^+$	$CD3^+CD4^+$	$CD3^+CD8^+$	$CD19^+CD5^-$	$CD3^-CD16^+CD56^+$	
1	F	53	2	−	155	93	49	123	3	Severe lung infection, MODS
2	M	79	18	−	388	251	56	63	56	Severe lung infection, MODS
3	F	46	2	+	1,900	1,400	410	1,150	370	Severe lung infection, MODS
4	F	44	12	+	444	257	161	NA	NA	Severe lung infection, RF
5	F	49	2.5	+	215	167	50	44	14	AIP, lung infection, RF
6	F	82	10	+	398	317	74	136	26	Lung cancer
7	F	68	24	+	385	80	269	62	30	Lung cancer

F female, *M* male, *MODS* multiple organ dysfunction syndrome, *RF* respiratory failure, *AIP* acute interstitial pneumonia, − negative, + positive, *NA* not available

use of immunosuppressive drugs was in fact positively correlated with low counts of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells.

In addition, lymphocyte subsets have been found to be affected by some internal characteristics of the disease, such as the level of disease activity. Aleksza et al. concluded that there were decreased percentages of T ($CD3^+$) lymphocytes and Tc ($CD8^+$) lymphocytes in active DM [3]. Another report provided evidence that peripheral blood lymphocyte subsets in patients with each type of IIM (PM/DM) were different [2]. In our study, we first selected previously untreated patients with active (aDM) and inactive (iDM) DM. We identified and compared the levels of peripheral blood lymphocyte subsets between the different groups. It was found that counts of T ($CD3^+$) cells, $CD4^+$ T cells, and $CD8^+$ T cells in the aDM group were all significantly lower than in the iDM and control groups. Counts of T cells, $CD4^+$ T cells, and $CD8^+$ T cells reached close to normal levels in iDM, with no statistically significant differences as compared to the control group. We also found that $CD3^+$ and $CD3^+CD4^+$ cell counts were significantly lower in DM before treatment, compared with after treatment. Percentages of B2 ($CD19^+CD5^-$) cells, NK ($CD3^-CD16^+CD56^+$) cells, and NK cell counts in both aDM and iDM were significantly lower than in controls. A previous study by Viguier et al. also reported that low peripheral blood T cells, $CD4^+$ T cells, and $CD8^+$ T cells were consistent findings in patients with active DM [9]. Takamure reported that the percentage of $CD8^+CD28^+/CD8^+$ T cells and the absolute number of $CD8^+CD28^+$ positive T cells was significantly lower in the patients with untreated active PM than in the patients with post-treated inactive PM and the healthy controls, speculating that the CD28 pathway plays a role in PM [10]. Due to the small number of newly diagnosed patients with PM, comparisons between active PM, inactive PM, and health control groups were not made. However, using multivariate logistic regression analysis, we further found that PM/DM total disease activity scores were positively correlated with decreased counts of T cells, $CD4^+$ T cells, and $CD8^+$ T cells, and negatively correlated with a high B2 cell count. We concluded that T cells, $CD4^+$ T cells, and $CD8^+$ T cells may be useful as a reference marker for evaluating disease activity, despite the unclear pathophysiology underlying low numbers of peripheral T lymphocytes. B2 cells, commonly referred as B-lymphocytes, are the main source of specific antibodies.

In our study, we found that B2 lymphocytes in patients with active DM and inactive DM were all higher than those in healthy controls, suggesting that humoral immunity may play a role in DM. Elevated B2 cells in patients with inactive DM may contribute to the continuous production of auto-antibodies. It was noted that B2 cell counts in iDM were significantly higher than in aDM although we do not have a good

Table 6 Risk factors for death in PM/DM

	B	S.E.[a]	χ_{Wald}^{2} [a]	OR (95%CI)[a]	*P* value[a]
Malignant tumor	4.882	2.127	5.271	131.9 (2.043, 8,518.9)	0.015
Count of $CD3^+CD8^+$ cells	−0.011	0.005	4.762	0.989 (0.979, 0.999)	0.029

S.E. standard error, *OR* odds ratio, *b* regression coefficient

[a] Statistical results obtained using logistic regression

explanation for this finding. In addition, multivariate analysis showed that low percentages of $CD4^+$ T cells were correlated with disease type (PM or DM), and that reduced numbers of $CD4^+$ T cells were more common in patients with DM, which may indicate different roles of $CD4^+$ T cells in the pathogenesis of PM and DM.

Furthermore, we found that low counts of peripheral T cells and a low percentage of $CD4^+$ T cells were associated with the presence of ILD in PM/DM. Kurasawa et al. examined the activation markers of T cells in bronchoalveolar lavage fluid (BALF) from patients with interstitial pneumonitis (IP) in DM/PM, and found that $CD25^+CD4^+$ T cells in BALF were significantly increased in IP in DM/PM as compared with those in controls, suggesting that activated Th1-type pulmonary T cells plays an important role in the mechanism of lung injury in DM/PM [11]. In our previous studies, our group also found that large infiltrates of T lymphocytes and minor infiltrates of B lymphocytes are found in lung tissue of autoimmune myositis animal models, with the degree of infiltration correlating to the levels of myositis disease activity and the degree of lung injury [12]. This suggests that T cell and B cell-mediated immune injury may be one mechanism of lung injury in autoimmune myositis models. We found that low numbers of peripheral T cells were associated with the presence of ILD in PM/DM, which may prove useful in determining the presence of clinical lung involvement.

In the follow-up period, seven deaths were recorded. The counts of T cells, $CD4^+$ T cells, and $CD8^+$ T cells in these patients at the time of enrollment were found to be significantly decreased. We subsequently were able to conclude by means of multivariate logistic regression analysis that a low $CD8^+$ T cell count at enrollment and the presence of malignancy are independent risk factors for death in PM/DM. Age, disease duration, ILD, auto-antibodies etc. were not independent risk factors for death in PM/DM.

So far, conclusions of studies analyzing peripheral blood lymphocytes in PM/DM have been controversial. Besides the differences in study population, patient number, and patient inclusion/exclusion criteria, these inconsistencies are likely also due to the fact that several investigators chose to use single-fluorescent-labeled antigen to analyze lymphocyte subsets [13, 14]. This technique may impact the levels of $CD4^+$ T cells and $CD8^+$ T lymphocytes, as some mononuclear cells can express CD8 and some NK cells can express CD4. Therefore, it is better to use multiple labeling methods, which are more accurate, to clinically detect lymphocyte subsets. In summary, T cell subsets in peripheral blood were significantly decreased in active DM, and were correlated with PM/DM total disease activity. The identification and quantification of T cell subsets may therefore serve as a reference maker for evaluating disease activity. Decreased T cells counts and percentages of $CD4^+$ T cells were correlated with the presence of ILD in PM/DM. T cell subsets may therefore also be useful in the comprehensive assessment of clinical lung involvement. Low counts of $CD8^+$ T lymphocytes significantly increase the risk of patient death, thereby predicting a poor outcome for patients with PM/DM.

Acknowledgments This work was supported by grants from the National Natural Science Foundation of China (number: 81072457).

Disclosures None.

References

1. Reed AM, Ernste F (2009) The inflammatory milieu in idiopathic inflammatory myositis. Curr Rheumatol Rep 11(4):295–301
2. Ishii W, Matsuda M, Shimojima Y, Sumida T, Ikeda S (2008) Flow cytometric analysis of lymphocyte subpopulations and Th1/Th2 balance in patients with polymyositis and dermatomyositis. Intern Med 47(18):1593–1599
3. Aleksza M, Szegedi A, Antal-Szalmas P, Irinyi B, Gergely L, Ponyi A, Hunyadi J, Sipka S, Zeher M, Szegedi G, Dankó K (2005) Altered cytokine expression of peripheral blood lymphocytes in polymyositis and dermatomyositis. Ann Rheum Dis 64 (10):1485–1489
4. Bohan A, Peter JB (1975) Polymyositis and dematomyositis (first of two parts). N Engl J Med 292(7):344–347
5. Bohan A, Peter JB (1975) Polymyositis and dermatomyositis (second of two parts). N Engl J Med 292(8):403–407
6. Sultan SM, Allen E, Oddis CV, Kiely P, Cooper RG, Lundberg IE, Vencovsky J, Isenberg DA (2008) Reliability and validity of the myositis disease activity assessment tool. Arthritis Rheum 58 (11):3593–3599
7. Dalakas MC, Hohlfeld R (2003) Polymyositis and dermatomyositis. Lancet 362(9388):971–982
8. Kojima T, Tanuma N, Aikawa Y, Shin T, Sasaki A, Matsumoto Y (1997) Myosin-induced autoimmune polymyositis in the rat. J Neurol Sci 151(2):141–148
9. Viguier M, Fouéré S, de la Salmonière P, Rabian C, Lebbe C, Dubertret L, Morel P, Bachelez H (2003) Peripheral blood lymphocyte subset counts in patients with dermatomyositis: clinical correlations and changes following therapy. Med (Baltimore) 82(2):82–86
10. Takamure M, Murata KY, Sugie K, Ueno S (2005) Altered expression of costimulatory molecules in patients with polymyositis. J Clin Neuromuscul Dis 6(4):149–152
11. Kurasawa K, Nawata Y, Takabayashi K, Kumano K, Kita Y, Takiguchi Y, Kuriyama T, Sueishi M, Saito Y, Iwamoto I (2002) Activation of pulmonary T cells in corticosteroid-resistant and -sensitive interstitial pneumonitis in dermatomyositis/polymyositis. Clin Exp Immunol 129(3):541–548
12. Zhao H, Wang GC (2011) Expression of TRAIL and its receptors in interstitial lung tissue of autoimmune myositis in rats [J]. Basic Clin Med 31(5):540–545
13. Eisenstein DM, O'Gorman MR, Pachman LM (1997) Correlations between change in disease activity and changes in peripheral blood lymphocyte subsets in patients with juvenile dermatomyositis. J Rheumatol 24(9):1830–1832
14. Ishida T, Matsumoto Y, Ohashi M, Sasaki R (1993) Analysis of lymphocyte subpopulations in peripheral blood in adult and juvenile cases of dermatomyositis. J Dermatol 20(1):30–34