Circulating T_{FH} cells is correlated with disease activity in anti-MDA5 antibody positive idiopathic inflammatory myopathies

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Abstract

Objective

Idiopathic inflammatory myopathies (IIM) are a group of disorders characterised by the production of autoantibodies and inflammatory infiltrates in the skeletal muscles. Follicular T helper (T_{FH}) cells are known to be crucial for B cell differentiation and autoantibody production in autoimmune diseases. The aim of this study was to investigate the involvement of T_{FH} cells in IIM.

Methods

Circulating T_{FH} cells in 44 IIM patients or 11 age- and gender-matched healthy controls (HCs) were measured by flow cytometry. ICOS, PD-1, active caspase-1 and Ki-67 expression in T_{FH} cells was examined. The correlations between the frequency of T_{FH} cells and clinical disease activities were also analysed.

Results

The frequency of T_{FH} cells was 16.6% in IIM patients with anti-melanoma differentiation-associated gene (MDA5) antibody compared to 10.6% and 12.9% in anti-MDA5 negative patients or HCs, respectively (both $p<0.05$). The frequency of T_{FH} cells was positively correlated with clinical disease activities: patient/parent's assessment VAS ($r=0.51$, $p<0.05$), physician's assessment VAS ($r=0.59$, $p<0.05$) and MYOACT scores (total systems: $r=0.62$, $p<0.05$; extramuscular system: $r=0.56$, $p<0.05$; pulmonary system, $r=0.55$, $p<0.05$). The percentage of PD-1^{high}ICOS^{high} T_{FH} cells was 3.68% in anti-MDA5 positive patients compared to 2.70% and 1.96% in anti-MDA5 negative patients or HCs, respectively (both $p<0.05$). The percentage of Ki-67 positive T_{FH} cells was 3.50% in anti-MDA5 positive patients compared to 2.36% and 1.76% in anti-MDA5 negative patients or HCs, respectively ($p<0.05$). Interestingly, active caspase-1 was significantly increased in T_{FH} cells in anti-MDA5 positive patients compared to the patients without anti-MDA5 or HCs (3.30% vs. 1.67% and 3.30% vs. 1.02%, both $p<0.001$).

Conclusion

These data suggest a role for T_{FH} cells in the pathogenesis of anti-MDA5 positive IIM and T_{FH} cells might serve as a disease biomarker for this subset of patients.

Key words

follicular T helper cells, idiopathic inflammatory myopathies, anti-melanoma differentiation-associated gene 5 antibody, caspase-1

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Introduction

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of muscle diseases, manifested as varying severity of muscle inflammation and extramuscular symptoms. The majority of IIM can be classified into polymyositis (PM), inclusion body myositis (IBM), amyopathic dermatomyositis (ADM) or dermatomyositis (DM) (1). Emerging evidence has discovered several pathogenic myositis-specific antibodies (MSAs), such as anti-TIF1-γ, NXP2, SAE, HMGCR and MDA5 (Melanoma differentiation-associated gene 5, MDA5) antibody (2-4). The specific types of autoantibodies are linked with different patterns of clinical manifestations and are helpful to classify IIM patients and to predict severe organ involvement and prognosis.

Follicular T helper (T_{FH}) cells are a specialised subset of $CD4^+$ T cells, which are located in the germinal centre. They professionally promote B cell differentiation into plasma cells and production of antibodies (5). Recent studies have reported a subset of $CXCR5^+CD4^+$ T cells in the circulation which share common developmental mechanisms, phenotypes and functional properties with T_{FH} cells (6). They are considered to be circulating memory compartment of T_{FH} lineage in the blood (6). Accumulating evidences demonstrate that T_{FH} cells were highly activated and expanded in autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS) and autoimmune thyroid disease (7-10).

Regarding the role of emerging pathogenic autoantibodies in the pathogenesis of IIM, an aberrant response might happen to T_{FH} cells in IIM. However, it is still unclear whether homeostatic state of T_{FH} cells are altered and how they contribute to the pathogenesis of IIM. Several studies reported conflicting results on the status of T_{FH} cells in peripheral blood of IIM patients. Espinosa-Ortega *et al*. found increased numbers of T_{FH} cells in 30 IIM patients while Sasaki *et al*. found fewer T_{FH} cells in 13 DM patients (11, 12), but neither of them discussed the characteristics of T_{FH} cells in the circulation according to the pattern of MSAs. In the present study, we aim to explore the frequency of T_{FH} cells and the potential involvement of T_{FH} cells in subgroups of IIM patients. We found that circulating T_{FH} cells were increased only in IIM patients with anti-MDA5 antibody and active caspase-1 was increased in T_{FH} cells from these patients. The increased frequency of T_{FH} cells was correlated with disease activities only in patients with anti-MDA5 antibody, indicating a positive role of T_{FH} cells in the pathogenesis of IIM with anti-MDA5 antibody.

Materials and methods

Patients

Forty-four hospitalised IIM patients fulfilled the Peter and Bohan diagnosis criteria (13) were recruited from the Department of Rheumatology, the First Affiliated Hospital, Sun Yat-Sen University. Patients with infection or pregnancy were excluded. In the subsequent analysis, all 44 patients also met 2017 EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies (1). Patients were recruited consecutively and were not selected for any characteristics including autoantibodies. Eleven age- and gender-matched healthy controls (HCs) were included in the present study. Demographic and clinical characteristics of the IIM patients and HCs are shown in Supplementary Table S1. This study was approved by the Institutional Ethical Committee of the First Affiliated Hospital, Sun Yat-Sen University. Informed consents were obtained from IIM patients and HCs.

Clinical parameters and clinical scales

MSAs (including anti-MDA5, anti-Ro-52, anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, anti-Mi-2α, anti-Mi-2β, anti-TIF1-γ, anti-NXP2, anti-SAE1, anti-Ku, anti-PM-Scl100, anti-PM-Scl75, anti-SRP) were detected by a commercially available line immunoassay (Euroline Myositis Profile, EUROIMMUN, Luebeck, Germany). Disease activities of myositis were evaluated using patient/parent's assessment of disease activity on a Visual Analogue Scale (VAS), physician's assessment

of disease activity on a VAS, the Myositis Disease Activity Visual Analogue Scales (MYOACT) and manual muscle test (MMT) (14). MYOACT is scored by extramuscular system (the sum of 6 individual organ systems including constitutional, cutaneous, skeletal, gastrointestinal, pulmonary, cardiac), muscular system and the total systems.

PBMC isolation and flow cytometry

Blood from 44 IIM patients and 11 HCs were drawn into tubes coated with heparin. Peripheral blood mononuclear cells (PBMCs) were isolated using density-gradient centrifugation on Ficoll-Paque™ PLUS (GE Healthcare, USA). Single cell suspensions were prepared for flow cytometry. For surface marker and FAM-FLICA caspase-1 staining, cells were stained with antibodies against CD4, CXCR5, ICOS, PD-1 (BioLegend, USA) and a FAM-FLICA caspase-1 probe (ImmunoChemistry Technologies) at 4°C for 30 minutes and then washed with PBS twice. For Foxp3 and Ki-67 staining, cells were fixed and permeabilised with a Foxp3 Staining Set (eBioscience, USA) and then stained for Foxp3 and Ki-67 (BioLegend, USA). Samples were analysed by flow cytometry with a CytoFLEX analyser (Beckman Coulter). FlowJo software (Tree Star, USA) was used for FAC data analysis.

Statistics

All data are presented as mean ± SEM. Statistical analysis was performed using GraphPad Prism 7. The differences were assessed by t-test, chi-square test or one-way ANOVA as appropriate. For correlation analysis, Spearman correlation coefficient was applied. Two-tailed $p<0.05$ was considered statistically significant.

Results

Clinical features of IIM patients

In the present study, 44 IIM patients and 11 HCs were included. The demographic characteristics of the patients were presented in Supplementary Table S1. There is no difference in age and gender between patients and HCs. Seventy-five percentages of IIM patients were diagnosed as DM and the remaining patients were PM.

To further analyse subgroups of IIM, we subdivided patients into two groups according to the pattern of MSAs. Among 44 IIM patients, 16 (36%) were positive for anti-MDA5 antibody (Ab) (Suppl. Table S2). The remaining patients were either negative or positive for other myositis specific autoantibodies as shown in Supplementary Table S3. Eight patients had two or more autoantibodies simultaneously and 8 were negative for the autoantibodies tested. Significantly higher prevalence of skin rash, including cutaneous ulceration, heliotrope sign and Gottron papules/sign were observed in anti-MDA5 Ab-positive patients. ADM, ILD and arthritis were more commonly seen in anti-MDA5-positive group. Anti-MDA5 positive patients exhibited significantly lower CK and LDH levels and lower leukocyte or lymphocyte counts (Suppl. Table S4). There were no statistically significant differences in fever, dyspnea, myalgia, cardiomyopathy, calcinosis, AST, ALT, ESR, CRP, ANA, ferritin and immunoglobulin between the two groups.

Frequency of circulating T_{FH} cells was increased in IIM patients with anti-MDA5 antibody

PBMCs from 44 IIM patients and 11 HCs were subjected to flow cytometry for analysis of T_{FH} cells. $CD4^+CXCR5^+Foxp3^-$ cells were considered to be circulating T_{FH} cells as reported previously (7). The frequency of $CD4^+CXCR5^+Foxp3^-$ T_{FH} cells was not changed in patients with IIM when compared to HCs. Further subgroup analysis showed that the frequency of T_{FH} cells was increased only in patients with anti-MDA5 antibody. The frequency of T_{FH} cells in patients without anti-MDA5 antibody was not statistically different to that in HCs (Fig. 1A-D).

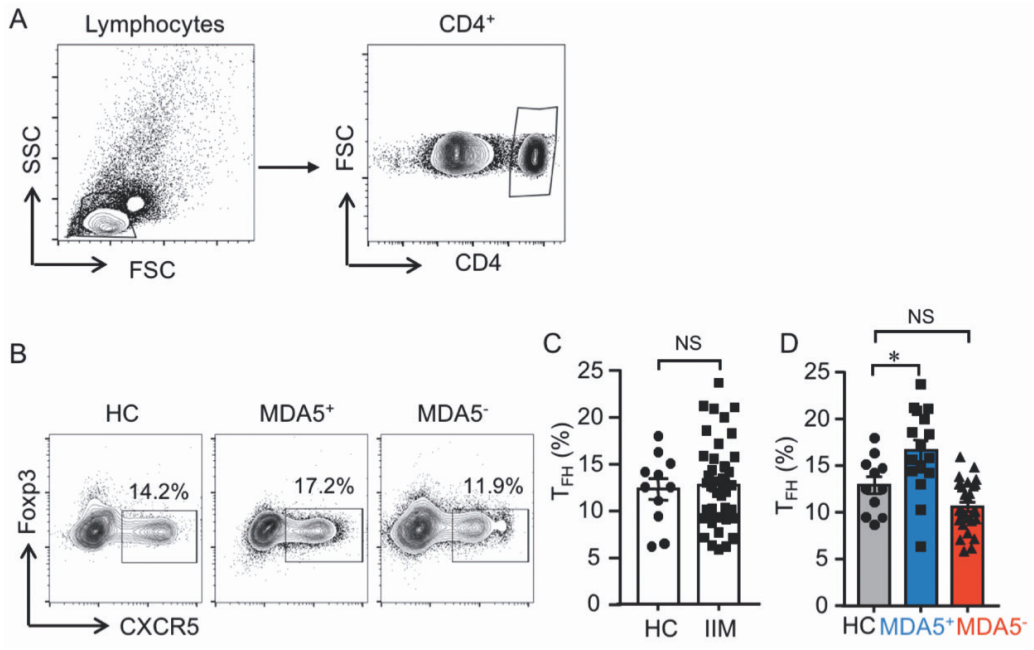

Fig. 1. Percentages of circulating T_{FH} cells in patients with IIM and HCs. PBMCs of patients with idiopathic inflammatory myopathies (IIM) and healthy controls (HCs) were analysed by flow cytometry. **A**: Gating strategy. Lymphocytes were gated on $CD4^+$ cells and further identified as circulating T_{FH} ($CD4^+CXCR5^+Foxp3^-$) cells. **B**, **D**: Representative contour plots and percentages of circulating T_{FH} among $CD4^+$ T cells in HCs (n=11), anti-$MDA5^+$ IIM patients (n=16) and anti-$MDA5^-$ IIM patients (n=28). **C**: Percentages of circulating T_{FH} cells among $CD4^+$ T cells in IIM patients (n=44) and HCs. NS: not significant. *$p<0.05$.

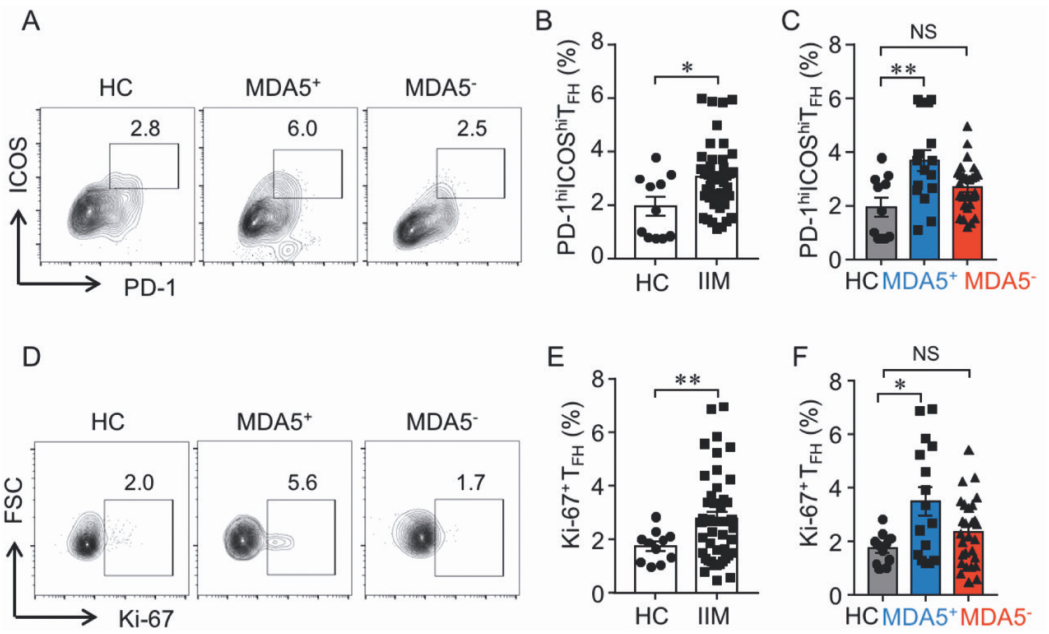

Fig. 2. Functional phenotypes of T_{FH} cells in IIM patients and HCs. PBMCs from IIM patients and healthy controls (HCs) were analysed by flow cytometry.
A-**C**: Representative contour plots and percentages of PD-$1^{high}ICOS^{high}$ cells gated on T_{FH} cells.
D-**F**: Representative contour plots and percentages of Ki-67^+ cells among T_{FH} cells.
NS: not significant.
*p<0.05, **p<0.01.

Circulating T_{FH} cells were activated in IIM patients with anti-MDA5 antibody

PD-$1^+ICOS^-CXCR5^+$ T cells represent resting and PD-$1^+ICOS^+CXCR5^+$ T cells represent recently activated memory T_{FH} cells (15, 16). We found that the percentage of PD-$1^{high}ICOS^{high}$ T_{FH} cells was increased in the circulation of IIM patients (Fig. 2A-B). However, the increased PD-$1^{high}ICOS^{high}$ T_{FH} cells was only observed in patients with anti-MDA5 antibody. There was no significant difference in the percentage of PD-$1^{high}ICOS^{high}$ T_{FH} cells between HCs and IIM patients without anti-MDA5 antibody (Fig. 2A, C). The expression of Ki-67, a marker of proliferation, was increased in T_{FH} cells from IIM patients, which was attributed to the increased expression of Ki-67 in T_{FH} cells from patients with anti-MDA5 antibody. We did not observe the difference in the expression of Ki-67 in T_{FH} cells from patients without anti-MDA5 antibody and HCs (Fig. 2D-F).

Inflammasome associated caspase-1 was activated in circulating T_{FH} cells in IIM patients with anti-MDA5 antibody

Recent findings suggest that IL-1 signalling is involved with T_{FH} cells in controlling B cell responses (17). The production of IL-1 is controlled by inflammasome associated caspase-1 activation (18). The activation of caspase-1 leads to the maturation and secretion of IL-1 (19). To assess the potential role of inflammasome associated caspase-1 activation in patients with IIM, a fluorescence-labeled inhibitor probe, which binds to intracellular active caspase-1 specifically (20), was used to measure levels of active caspase-1 in T_{FH} cells. The data showed that the percentage of active caspase-1^+ T_{FH} cells was increased in patients with IIM when compared to that from HCs. However, increased active caspase-1^+ T_{FH} cells were only observed in patients with anti-MDA5 antibody. No difference was found between patients without anti-MDA5 antibody and HCs (Fig. 3A-C).

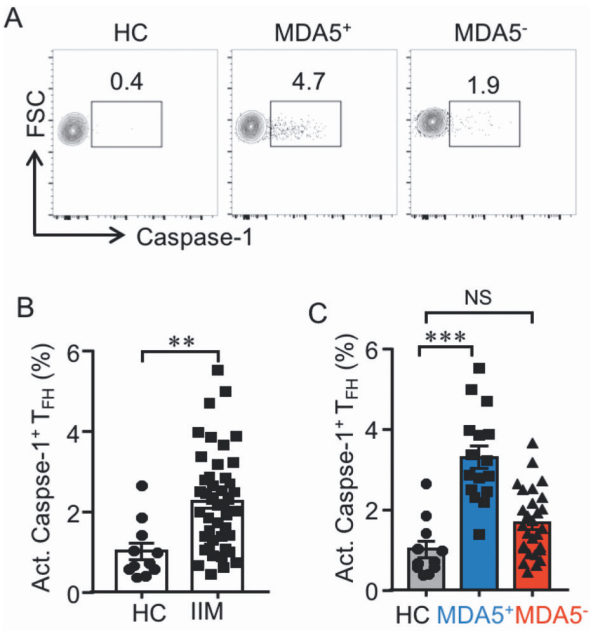

Fig. 3. Activation of inflammasome associated caspase-1 in T_{FH} cells in IIM patients. PBMCs from IIM patients and healthy controls (HCs) were analysed by flow cytometry. **A**, **C**: Representative dot plots and frequencies of active caspase-1^+ cells among T_{FH} cells in anti-MDA5^+, anti-MDA5^- IIM patients and HCs.
B: Percentages of active caspase-1^+ cells in T_{FH} cells in IIM patients and HCs.
NS: not significant.
**p<0.01, ***p<0.001.

Circulating T_{FH} cells were correlated with disease activity in IIM patients with anti-MDA5 antibody

To investigate whether T_{FH} cells contributed to the disease in patients with IIM, we further analysed the association between the frequency of T_{FH} cells and diseases activity. Disease activity was evaluated according to patient/parent's assessment VAS, physician's as-

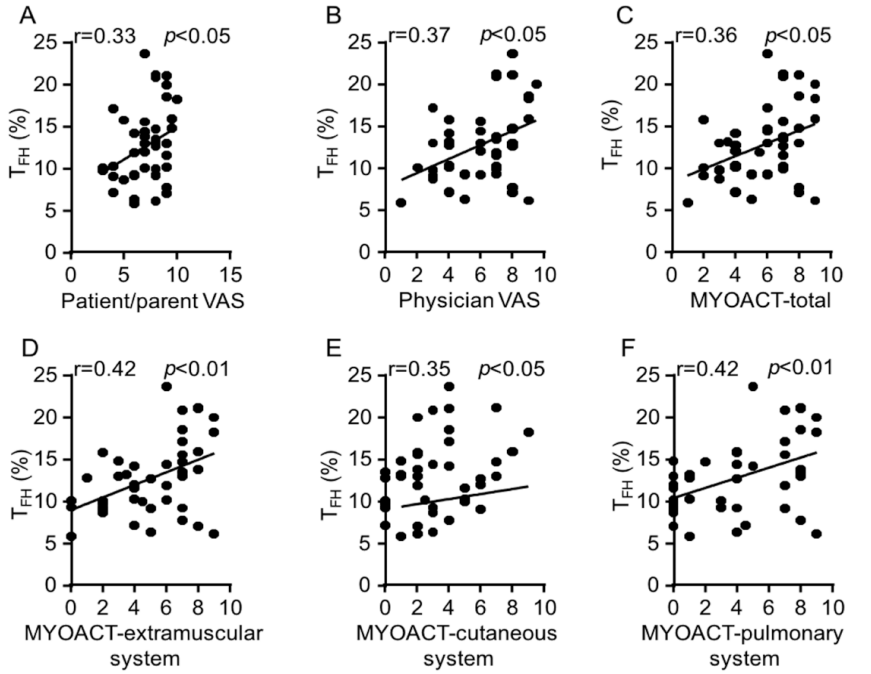

Fig. 4. Correlation between the percentage of T_{FH} cells with disease activities in IIM patients.
A-**F**: IIM disease activity was measured as indicted. Correlation between the percentage of T_{FH} with Patient/parent VAS (**A**), Physician VAS (**B**), MYOACT-total (**C**), MYOACT-extramuscular system (**D**), MYOACT-cutaneous system (**E**) and MYOACT-pulmonary system (**F**) was shown. Patient/parent VAS, patient/parent global activity assessment using a visual analogue scale; Physician VAS, physician global activity assessment using a visual analogue scale; MYOACT, myositis disease activity assessment visual analogue scales.

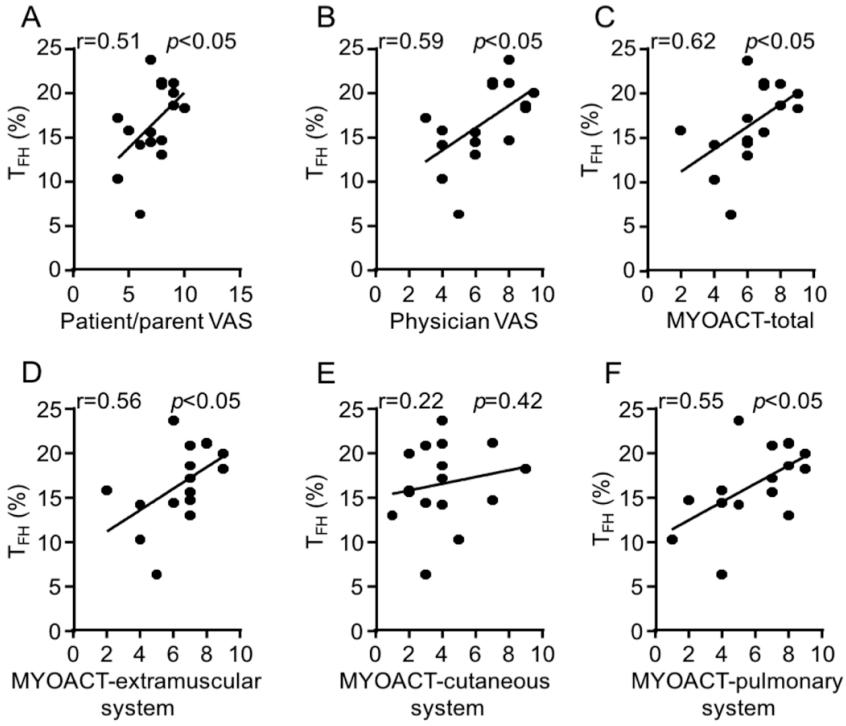

Fig. 5. Correlation between the percentage of T_{FH} cells with disease activities in patients with anti-MDA5 antibody.
A-**F**: Correlation between the percentage of T_{FH} with Patient/parent VAS (**A**), Physician VAS (**B**), MYOACT-total (**C**), MYOACT-extramuscular system (**D**), MYOACT-cutaneous system (**E**) and MYOACT-pulmonary system (**F**) was shown. Patient/parent VAS, patient/parent global activity assessment using a visual analogue scale; Physician VAS, physician global activity assessment using a visual analogue scale; MYOACT, myositis disease activity assessment visual analogue scales.

sessment VAS, MYOACT scores and MMT. We found that the frequencies of T_{FH} cells were significantly correlated with patient/parent's assessment VAS, physician's assessment VAS (Fig. 4A-B). Similar results were also observed regarding MYOACT-total, MYOACT-extramuscular, MYOACT-cutaneous and MYOACT-pulmonary systems (Fig. 4C-F).

From the data above, circulating T_{FH} cells seemed to be involved in IIM in patients with positive anti-MDA5 antibody only. To further confirm the contribution of circulating T_{FH} cells in subsets of IIM patients, we analysed the correlations between circulating T_{FH} cells in IIM subgroup of patients with or without anti-MDA5 antibody. The percentage of T_{FH} cells was correlated with disease activity scales of Patient/parent VAS, Physician VAS (Fig. 5A-B) in patients with anti-MDA5 antibody. The percentage of T_{FH} cells was also correlated with MYOACT-total, MYOACT-extramuscular and MYOACT-pulmonary systems in patients with positive anti-MDA5 antibody (Fig. 5C, D, F). However, there is no correlation between T_{FH} cells and MYOACT-cutaneous system in patients with anti-MDA5 antibody (Fig. 5E). On the other hand, in patients without anti-MDA5 antibody, we did not find correlations between the frequency of T_{FH} cells and disease activity scales (Fig. 6).

Discussion

Disturbances of T_{FH} cells have been reported to contribute to the pathogenesis of autoimmune diseases (7-10). Circulating $CXCR5^{+}CCR7^{low}PD\text{-}1^{high}CD4^{+}$ T cells is considered as the precursor cells of T_{FH} cells in circulation (21). It was found aberrantly expanded in IIM and was shown to significantly correlate with disease activity (21). However, evidences regarding the roles of T_{FH} cells in the pathogenesis of IIM are conflicting (11, 12). In the present study, we did not find any significant difference in the percentages of T_{FH} cells between the IIM group and the healthy controls. Nevertheless, when subclass analysis was performed according to positivity of MDA5-antibody, we found a significantly higher percentage of T_{FH} cells

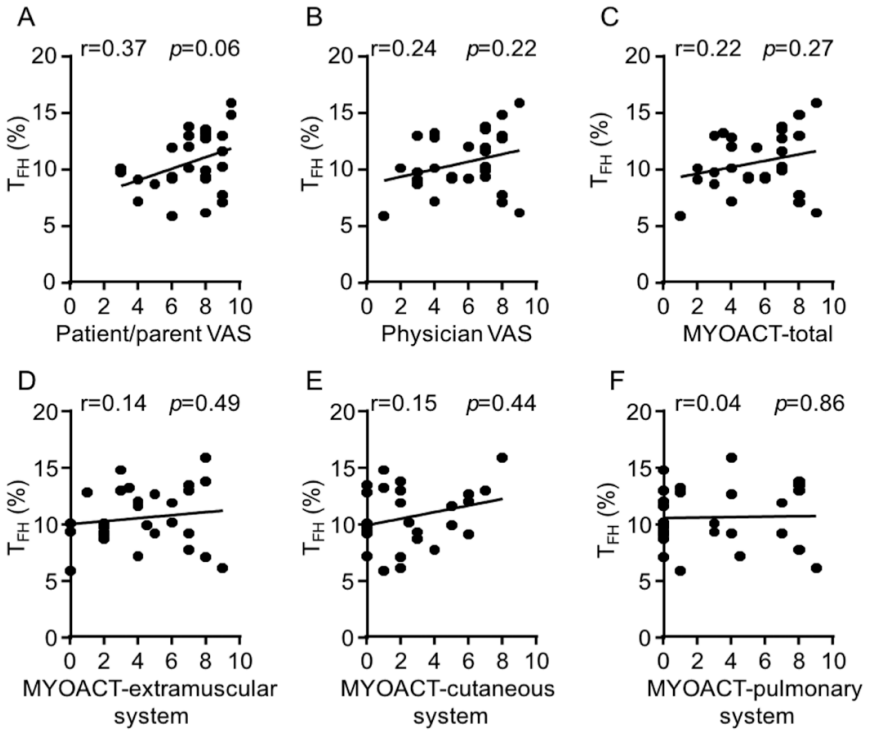

Fig. 6. Correlation between the percentage of T_{FH} cells with disease activities in IIM patients without anti-MDA5 antibody.
A-**F**: Correlation between the percentage of T_{FH} with Patient/parent VAS (**A**), Physician VAS (**B**), MYOACT-total (**C**), MYOACT-extramuscular system (**D**), MYOACT-cutaneous system (**E**) and MYOACT-pulmonary system (**F**) was shown. Patient/parent VAS, patient/parent global activity assessment using a visual analoue scale; Physician VAS, physician global activity assessment using a visual analogue scale; MYOACT, myositis disease activity assessment visual analogue scales.

in the antibody-positive patients compared to the MDA5-antibody negative patients, suggesting that T_{FH} cells might have a particular role in the pathogenesis in IIM patients with anti-MDA5 antibody.

Anti-MDA5 antibody is a recently described MSA. It is exclusively present in 20–30% of DM patients (22). The percentages (36%) of anti-MDA5 antibodies in this cohort is higher than the previous reports. This is probably because our hospital is a tertiary institution receiving sicker referral patients and anti-MDA5 is associated with disease severity. MDA5 is a cytoplasmic retinoic acid-inducible gene-1-like receptor, which senses RNA with a helicase domain and subsequently induces the expression of type 1 interferon (T1-IFN) and pro-inflammatory cytokines (23). Anti-MDA5 antibody, originally called anti-CADM-140 antibody, is specific for DM and closely associated with the life-threatening rapidly progressive interstitial lung disease (RP-ILD) (24). Anti-MDA5 antibody-positive DM patients are also characterised by unique skin lesions, especially the cutaneous ulceration, and a lower rate of myositis (25). In this study, ILD, ADM and mucocutaneous lesions were more commonly seen in IIM patients with anti-MDA5 antibody.

Antibody production by B cells depend on the help provided by $CD4^+$ T cells largely (26), more specifically by a $CD4^+$ T cell subset T_{FH} cells (27). In the present study, we found that circulating T_{FH} cells were increased in IIM patients with anti-MDA5 antibody. Higher expression of PD-1 and ICOS as well as Ki-67 on T_{FH} cells was found in patients with anti-MDA5 antibody. $PD\text{-}1^+ICOS^+CXCR5^+$ T_{FH} cells display a phenotype of effector memory T cells (6). ICOS is a molecule that is essential for T_{FH} cell generation. In addition, ICOS itself acts as an important co-stimulatory molecule to induce the production of IL-21 by T_{FH} cells, which is critical for B cell response (28). ICOS expression was increased in T_{FH} cells from patients with anti-MDA5 antibody, indicating that T_{FH} cells from patients with anti-MDA5 antibody could provide stronger help to B cells and that might contribute to the production of anti-MDA5 autoantibody.

IL-1 signalling is involved in the function of T_{FH} cells (17). Mice deficient in the receptor for IL-1 showed a significant decrease in T_{FH} cells compared to that in wild-type mice (29). The maturation and production of IL-1 are controlled by inflammasome associated caspase-1 activation (19, 30), demonstrating the association of inflammasome associated caspase-1 activation with T_{FH} cells. We found the active caspase-1 was increased in T_{FH} cells from IIM patients with anti-MDA5 antibody. It has been shown that caspase-1 inhibitor inhibited humoral immunity response by suppressing T_{FH} cells in an animal model of experimental autoimmune myasthenia gravis (31). The increased activation of caspase-1 in T_{FH} cells might form a positive feedback loop that could affect the proliferation of the T_{FH} cells, since we observed an increase of Ki-67 expression in these cells. Recently, data have shown the involvement of caspase-1 in the differentiation of Th1 and Th17 cells (32, 33). Our results suggest that the activation of caspase-1 enhances the differentiation of the T_{FH} cells in patients with anti-MDA5 antibody and targeting NLRP3 inflammasome might have therapeutic implication for this disease. However, further studies are needed to confirm this speculation.

In conclusion, percentage of circulating T_{FH} cells is increased and correlated with disease activities in IIM patients with positive anti-MDA5 antibody. These findings suggest a role for T_{FH} cells in the pathogenesis of anti-MDA5 positive IIM and T_{FH} cells might serve as a biomarker for this subset of patients.

References

1. LUNDBERG IE, TJÄRNLUND A, BOTTAI M *et al.*: 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis* 2017; 76: 1955-64.
2. MAMMEN AL: Autoimmune myopathies: autoantibodies, phenotypes and pathogenesis. *Nat Rev Neurol* 2011; 7: 343-54.

3. DAMOISEAUX J, VULSTEKE J-B, TSENG C-W *et al*.: Autoantibodies in idiopathic inflammatory myopathies: Clinical associations and laboratory evaluation by mono- and multispecific immunoassays. *Autoimmun Rev* 2019; 18: 293-305.
4. TRIPOLI A, MARASCO E, COMETI L *et al*.: One year in review 2019: idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2020; 38: 1-10.
5. UENO H, BANCHEREAU J, VINUESA CG: Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol* 2015; 16: 142-52.
6. SCHMITT N, BENTEBIBEL S-E, UENO H: Phenotype and functions of memory Tfh cells in human blood. *Trends Immunol* 2014; 35: 436-42.
7. XU B, WANG S, ZHOU M *et al*.: The ratio of circulating follicular T helper cell to follicular T regulatory cell is correlated with disease activity in systemic lupus erythematosus. *Clin Immunol* 2017; 183: 46-53.
8. WANG J, SHAN Y, JIANG Z *et al*.: High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. *Clin Exp Immunol* 2013; 174: 212-20.
9. BROKSTAD KA, FREDRIKSEN M, ZHOU F *et al*.: T follicular-like helper cells in the peripheral blood of patients with primary Sjogren's syndrome. *Scand J Immunol* 2017 Jun 8 [Online ahead of print].
10. ZHU C, MA J, LIU Y *et al*.: Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. *J Clin Endocrinol Metab* 2012; 97: 943-50.
11. ESPINOSA-ORTEGA F, GOMEZ-MARTIN D, SANTANA-DE ANDA K, ROMO-TENA J, VILLASENOR-OVIES P, ALCOCER-VARELA J: Quantitative T cell subsets profile in peripheral blood from patients with idiopathic inflammatory myopathies: tilting the balance towards proinflammatory and pro-apoptotic subsets. *Clin Exp Immunol* 2015; 179: 520-8.
12. SASAKI H, TAKAMURA A, KAWAHATA K *et al*.: Peripheral blood lymphocyte subset repertoires are biased and reflect clinical features in patients with dermatomyositis. *Scand J Rheumatol* 2019; 48: 225-9.
13. BOHAN A, PETER JB: Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975; 292: 344-7.
14. MILLER FW: New approaches to the assessment and treatment of the idiopathic inflammatory myopathies. *Ann Rheum Dis* 2012; 71 (Suppl. 2): i82-5.
15. HE J, TSAI LM, LEONG YA *et al*.: Circulating precursor CCR7(lo)PD-1(hi) CXCR5$^{(+)}$ CD4$^{(+)}$ T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. *Immunity* 2013; 39: 770-81.
16. LOCCI M, HAVENAR-DAUGHTON C, LANDAIS E *et al*.: Human circulating PD-1^{+}CXCR3^{-}CXCR5^{+} memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* 2013; 39: 758-69.
17. RITVO PG, KLATZMANN D: Interleukin-1 in the response of follicular helper and follicular regulatory T cells. *Front Immunol* 2019; 10: 250.
18. LATZ E, XIAO TS, STUTZ A: Activation and regulation of the inflammasomes. *Nat Rev Immunol* 2013; 13: 397-411.
19. FRANCHI L, EIGENBROD T, MUNOZ-PLANILLO R, NUNEZ G: The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol* 2009; 10: 241-7.
20. DOITSH G, GALLOWAY NL, GENG X *et al*.: Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; 505: 509-14.
21. ZHANG L, LI W, CAI Y, LIU X, PENG Q, LIANG L: Aberrant expansion of circulating CD4$^{(+)}$ CXCR5$^{(+)}$ CCR7(lo) PD1(hi) Tfh precursor cells in idiopathic inflammatory myopathy. *Int J Rheum Dis* 2020; 23: 397-405.
22. KANG EH, NAKASHIMA R, MIMORI T *et al*.: Myositis autoantibodies in Korean patients with inflammatory myositis: anti-140-kDa polypeptide antibody is primarily associated with rapidly progressive interstitial lung disease independent of clinically amyopathic dermatomyositis. *BMC Musculoskelet Disord* 2010; 11: 223.
23. KATO H, TAKEUCHI O, SATO S *et al*.: Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 2006; 441: 101-5.
24. SATO S, HIRAKATA M, KUWANA M *et al*.: Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. *Arthritis Rheum* 2005; 52: 1571-6.
25. KURTZMAN DJ, VLEUGELS RA: Anti-melanoma differentiation-associated gene 5 (MDA5) dermatomyositis: A concise review with an emphasis on distinctive clinical features. *J Am Acad Dermatol* 2018; 78: 776-85.
26. CROTTY S: T follicular helper cell differentiation, function, and roles in disease. *Immunity* 2014; 41: 529-42.
27. UENO H, BANCHEREAU J, VINUESA CG: Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol* 2015; 16: 142-52.
28. BENTEBIBEL SE, SCHMITT N, BANCHEREAU J, UENO H: Human tonsil B-cell lymphoma 6 (BCL6)-expressing CD4^{+} T-cell subset specialized for B-cell help outside germinal centers. *Proc Natl Acad Sci USA* 2011; 108: E488-97.
29. DOLENCE JJ, KOBAYASHI T, IIJIMA K *et al*.: Airway exposure initiates peanut allergy by involving the IL-1 pathway and T follicular helper cells in mice. *J Allergy Clin Immunol* 2018; 142: 1144-58, e8.
30. MARIATHASAN S, NEWTON K, MONACK DM *et al*.: Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 2004; 430: 213-8.
31. WANG CC, ZHANG M, LI H *et al*.: Caspase-1 inhibitor regulates humoral responses in experimental autoimmune myasthenia gravis via IL-6- dependent inhibiton of STAT3. *Neurosci Lett* 2017; 656: 169-76.
32. ARBORE G, WEST EE, SPOLSKI R *et al*.: T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4^{+} T cells. *Science* 2016; 352: aad1210.
33. GAO Y, DEASON K, JAIN A *et al*.: Transcriptional profiling identifies caspase-1 as a T cell-intrinsic regulator of Th17 differentiation. *J Exp Med* 2020; 217: e20190476.