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Short Communication

Mass cytometry reveals an impairment of B cell homeostasis in anti-synthetase syndrome

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ABSTRACT

Recent data suggest the implication of T, B and NK cells in the anti-synthetase syndrome (ASyS); nevertheless their role and activation states are poorly described.

We performed deep immune-profiling using 37 markers on peripheral blood cells from 10 ASyS patients versus 17 healthy donors (HD) and 26 myositis control patients.

We show decreased percentages of memory B cells in ASyS patients (mean ± SEM: ASyS = 13 ± 3%, HD = 37 ± 4% and 'myositis controls' = 32 ± 3), counterbalanced by increased percentages of naïve B cells. Interestingly, perifascicular infiltrations of memory B cells within muscle biopsies of ASyS patients suggest that they niche within the muscle.

1. Introduction

The anti-synthetase syndrome (ASyS) is an autoimmune disorder characterized by the presence of myositis, interstitial lung disease, Raynaud's phenomenon, arthritis and antibodies directed against amino-acyl-t-RNA synthetases (Benveniste et al., 2016; Marguerie et al., 1990). The progressive pulmonary disease observed in ASyS patients leads to poor prognosis. Approximately 80% of ASyS patients present antibodies directed against the histidyl t-RNA synthetase (Jo1) (Benveniste et al., 2007).

In patients presenting anti-Jo1 antibodies, antibody titers have been associated with disease activity (Stone et al., 2007). Immunization of mice with purified murine Jo1 peptides leads to the generation of activated T and B cells infiltrating the muscles and lungs, and directed against species-specific Jo1 epitopes (Katsumata et al., 2007). More recently, our group observed a differentiated NK cell profile in active ASyS anti-Jo1 patients (CD57+ NK cells), accompanied by substantial infiltrations of NK cells within the lungs of patients compared to healthy donors (Hervier et al., 2016). Altogether, these studies suggest the pathogenic role of Jo1 and anti-Jo1 antibodies, and the implication of B, T and NK cells in the physiopathology of anti-Jo1 ASyS.

Cytometry by time-of-flight (CyTOF) is a powerful single-cell immune profiling tool, which allows the simultaneous analysis of > 37 markers using metal-conjugated antibodies (Dzangué-Tchoupou et al., 2019). This tool is of particular interest in the discovery of novel cell-populations and biomarker identification. Associated with barcoding using palladium isotopes, CyTOF allows the identification of subtle differences within populations and different experimental groups (Dzangué-Tchoupou et al., 2018).

The aim of this study was to identify specific cell populations potentially involved in the physiopathology of anti-Jo1 ASyS.

2. Material and methods

2.1. Ethical approval

Approval for this study was obtained from the *Comité Consultatif sur le Traitement de l'Information en matière de la Recherche dans le domaine de la Santé* (CCTIRS) France (N°14.323) and all patients and healthy controls signed a written informed consent.

2.2. Human samples

In an attempt to identify populations, which could be implicated in

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Table 1
Clinical characteristics of active ASyS anti-Jo1 patients, active myositis control patients and healthy donors.

Disease	sIBM	IMNM anti-HMGCR	IMNM anti-SRP	ASS anti-Jo1	HD
n =	10	9	7	10	17
Age (years)	59.3 ± 2	44.1 ± 6	52.3 ± 8	47.8 ± 4	51.5 ± 3
Female percentage	61.1	77.7	57.1	69.2	na
Disease duration (years)	9 ± 1	8 ± 3	3.2 ± 3	2.5 ± 1	na
CK level (IU/L)	453 ± 154	3977 ± 1670	3430 ± 888	2816 ± 1141	na
MMT8	132.3 ± 5	127.9 ± 8	109.9 ± 10	134.2 ± 5	na
PGA	na	7.2 ± 0.4	7.7 ± 0.4	7.2 ± 0.5	na
Treated/untreated patients	0/18	1*/8	3**/4	1$/9	na
Treatment duration (± SEM)	0	4 years	4 ± 3 months	2 years	na

CK = creatine phosphokinase.
MMT8 = manual muscle testing 8.
PGA = physician global assessment.
CT = oral corticosteroid.
IVIG = intra veinous Immunoglobulin.
AZT = azathioprine.
MTX = methotrexate.
na = not applicable.
* This patient received CT (6 mg).
** For these treated patients, one received CT (30 mg), the other CT (50 mg) and the last IVIG/AZT (150 mg).
$ This patient received CT (5 mg) and MTX (20 mg/week).

the physiopathology of anti-Jo1 ASyS ($n = 10$), we screened their peripheral blood mononuclear cells (PBMC) using 36 markers by CyTOF barcoding. Firstly, mass cytometric data from ASyS patients was compared to those of 10 healthy donors. Next, populations of interest were characterized in comparison to a pool of 26 active myositis 'control' patients (MC) comprised of well-defined groups of myositis patients (inclusion body myositis (IBM, $n = 10$), anti-3-hydroxy-3-methylglutaryl coA reductase myopathy (HMGCR, $n = 9$) and anti-signal recognition particle myopathy (SRP, $n = 7$) and a total number of 17 healthy controls (HC). Anti-Jo1 patients and myositis control patients were diagnosed according to internationally accepted criteria (Allenbach et al., 2018; Lloyd et al., 2014). All these myositis patients were active and mostly untreated Table 1.

2.3. Mass cytometric experiments

Barcoding, mass cytometric staining, data acquisition and data preprocessing (gating out of beads and dead cells) were performed as previously described in a technical research article (Dzangué-Tchoupou et al., 2018). The list of antibodies used is available in Supplementary Table 1.

2.4. Data analysis and statistics

Using the 'Citrus' algorithm available in the Cytobank cloud-based platform, we performed automated identification of stratifying signatures in cellular populations. Thus, in order to identify main populations of interest, 'Citrus' was applied on 10 anti-Jo1 ASyS patients vs. 10 healthy donors.

The next step was to characterize populations of interest between anti-Jo1 ASyS patients, HC and MC in detail. For that, main cell populations such as B, T and NK cells were exported automatically using automated cell clustering using the "spanning-progression tree of density-normalized events" (SPADE) algorithm (available in the Cytobank cloud-based platform) for downstream analysis.

Data was further analyzed using supervised approaches such as dot plots and histograms. Statistics was performed using Graphpad prism software version 6. We used Shapiro-Wilk normality test to verify the distribution of quantitative data amongst the groups compared. In-text results are represented as mean ± SEM.

2.5. Immunostainings

Muscle biopsies from two anti-Jo1 ASyS were stained using anti-CD27 (cyanine 3; red), anti-CD20 (Alexafluor 488; green) and 4′,6-diamidino-2-phenylindole (DAPI; blue) to stain the nuclei.

3. Results

Mean ages of anti-Jo1 patients, HC and MC were respectively, 48 ± 4, 52 ± 3 and 52 ± 2 years (Table 1). The Citrus algorithm identified an increase in the expressions of CD57 within NK cells (CD56+ CD16+), IgD within B cells (CD19+) and CD38 within CD8 T cells (CD3+ CD8+) in ASyS patients ($n = 10$) compared to healthy donors (n = 10) (Supplementary Fig. 1).

Next, we aimed at characterizing these cell populations between ASyS patients (n = 10), HC ($n = 17$) and MC ($n = 26$) in detail. For that, NK, B and CD8+ T cells were exported from automated SPADE trees for downstream analysis. Concerning NK cells, we show an increase in differentiated CD57+ NK cells in anti-Jo1 patients: anti-Jo1 = 64 ± 4% vs (HC = 49 ± 3%, $p = .07$ and MC = 46 ± 4%, $p = .01$) (Supplementary Fig. 2). Considering that this observation was previously reported (Hervier et al., 2016), we decided to focus on B cells and CD8+ T cells.

We observed a decrease in the frequency of memory B cells (CD19+ CD27+) in anti-Jo1 patients: anti-Jo1 = 13 ± 3% vs (HC = 37 ± 4%, $p < .0001$ and MC = 32 ± 3%, $p = .001$), counterbalanced by an increase in the frequency of naive B cells (CD19+ CD27-): anti-Jo1 = 86 ± 3% vs (HC = 61 ± 4%, $p < .0001$ and MC = 66 ± 3, $p = .001$) (Fig. 1). We did not observe changes in the frequency of CD95+ or HLA-DR+ B cell populations between anti-Jo1 ASyS patients in comparison to control groups (Fig. 1).

Concerning CD8+ T cells, the frequency of CD38+ CD8+ T cells was increased only in control myositis patients compared to healthy controls. Nevertheless, activated HLA-DR+ CD8 T cells were increased in all myositis groups compared to healthy controls: HC = 2 ± 0.3% vs (anti-Jo1 = 5 ± 1%, $p = .02$ and MC = 4 ± 0.5, $p = .01$).

Finally, immunostainings performed on skeletal muscle biopsies from anti-Jo1 patients revealed the presence of perifascicular infiltrates of CD20+ CD27+ memory B cells (Fig. 2). This data suggest that memory B cells niche within the muscle in anti-Jo1 ASyS patients. We performed additional immunostains of CD27 and CD20 on patients with dermatomyositis, patients with sIBM, patients with IMNM and with

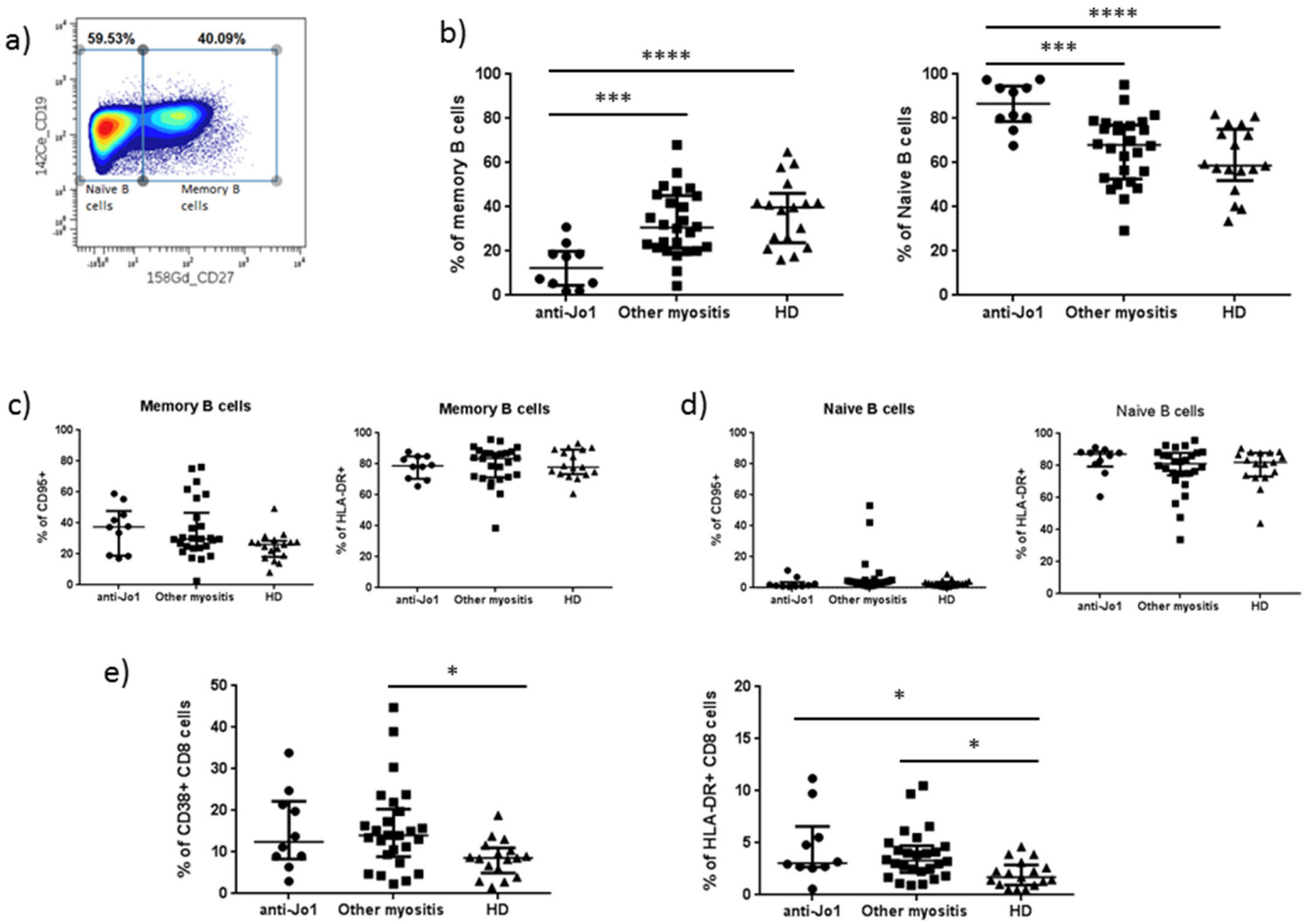

Fig. 1. Impairment of the peripheral B cell compartment in anti-Jo1 patients.
Automated cell clustering was performed using the SPADE algorithm available on cytobank. Next nodes corresponding to B cells were exported for downstream analyses. Here, we describe the percentages of B cell populations amongst patients presenting anti-Jo1 antibodies ($n = 13$), other myositis patients [$n = 26$: IBM ($n = 10$), anti-SRP myopathy ($n = 7$) and anti-HMGCR myopathy ($n = 9$)] and age-matched healthy donors ($n = 23$). **a)** dot plots showing the gating strategy used for naïve and memory B cells, **b)** comparison of the percentages of naïve and memory B cells between the three groups, **c)** percentages of CD95+ and HLA-DR+ memory B cells **d)** percentages of CD95+ and HLA-DR+ naïve B cells and **e)** immunostainings on muscle biopsies from two ASyS-Jo1+ patients showing memory CD20+ (AF488)/CD27+ (Cy3) cells around muscle fibers.
Statistics was performed with Graphpad Prism version 6. Shapiro-Wilk normality test was used to verify the distribution of quantitative data. Next, we performed comparisons using either one-way ANOVA test with Bonferroni correction or Kruskal-Wallis test with Dunn's correction ($*p < .05$, $**p < .001$, $***p < .0001$ and $****p < .00001$).

anti-Jo1 Abs (Supplementary Fig. 3). Results show that, while skeletal muscle biopsies from patients with IMNM and sIBM lack infiltrating memory B cells (CD20+ CD27+), DM and anti-Jo1 patients show infiltrating memory B cells. The presence of memory B cells in DM biopsies is in line with the literature (Radke et al., 2018).

4. Discussion

Despite the presence of specific auto-antibodies in anti-Jo1 ASyS patients, the role of B cells in the course of the disease is poorly described. Using mass cytometry, we show that within a homogenous group of patients presenting anti-Jo1 antibodies, there is an impairment

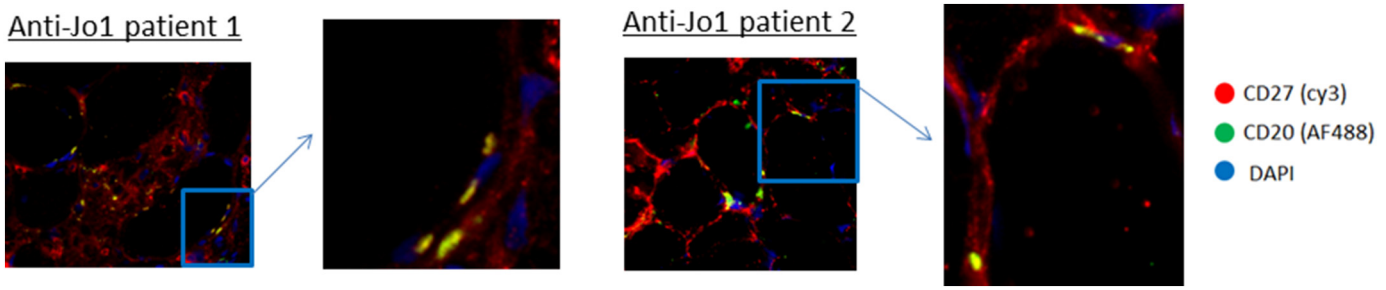

Fig. 2. Detection of memory B cells within muscle biopsies of anti-Jo1 patients.
Immunostainings on muscle biopsies from two ASyS-Jo1+ patients showing memory CD20+ (AF488)/CD27+ (Cy3) cells around muscle fibers and DAPI.

of the B cell compartment in comparison to control groups (healthy controls and a pool of three other sub-groups of myositis patients). We show that memory B cells are decreased in peripheral blood of anti-Jo1 ASyS patients compared to control groups. Unfortunately, we do not have access to PBMC from untreated patients with other anti-synthetase antibodies. In addition, we show the presence of memory B cells within the muscle of anti-Jo1 ASyS patients, which is one of the organs targeted by the disease. The dysregulation of B cell homeostasis in anti-Jo1 patients is unexpected, since neither other myositis patients presenting myositis specific autoantibodies (anti-HMGCR and anti-SRP myopathies) nor IBM patients present this impairment. This observation suggests the possibility that antigen-specific memory B cells home to target organs such as muscle, where they carry out effector functions.

We also observed an increase in the frequency of differentiated CD57+ NK cells in anti-Jo1 ASyS patients compared to control groups, in line with previous published data (Hervier et al., 2016).

In line with recent published data which observed an expansion of CD57+ CD8+ T cells in sIBM patients compared to HC or other myositis patients (Dzangué-Tchoupou et al., 2019; Greenberg et al., 2016), here we show that this cell population increases in the pool of 'myositis control' patients compared to healthy controls, particularly in sIBM patients (Supplementary Fig. 4). Nevertheless, this CD57+ CD8+ T cell population is not increased in ASyS anti-Jo1 patients.

Katsumata et al. (2007) showed that immunization of mice with purified murine Jo1 peptides leads to the generation of activated T and B cells infiltrating the muscles and lungs, and directed against species-specific Jo1 epitopes. In addition, our group (Hervier et al., 2016) observed a differentiated NK cell profile in active ASyS anti-Jo1 patients, accompanied by important infiltrations of NK cells within the lungs of patients compared to healthy donors. Based on these studies, the implication of B, T and NK cells is very likely in the antisynthetase syndrome. In this study, we show an impairment of B cells homeostasis and confirm the immune NK cell phenotype described previously specifically in antisynthetase syndrome patients and not in other myositis patients. Nevertheless, the presence of activated CD8+ T cells was also observed in sIBM and thus is not specific to antisynthetase syndrome myositis. Altogether, amongst 4 groups of myositis patients, we show an impairment of B cell homeostasis and an activated NK cell immune profile specific to ASyS anti-Jo1.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2019.04.014.

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