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Truncated isoforms of GPSM2 containing the GoLoco motif region promote CD4$^+$ T-cell migration in SLE

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ABSTRACT

Objective SLE is an autoimmune disease with a complex pathogenesis. T-cell infiltration into organs contributes to inflammation and organ damage in SLE. Recently, G-protein signalling modulator 2 (GPSM2) has been shown to be implicated in T-cell migration.

Methods We analysed the expression levels of GPSM2 and of a truncated isoform of GPSM2 containing the GoLoco motif region in CD4$^+$ T cells from patients with SLE and from healthy individuals by western blot. In a next step, we studied the role of the truncated GPSM2 isoform using a CD4$^+$ T-cell migration assay.

Results Our experiments revealed comparable levels of GPSM2 in CD4$^+$ T cells from patients with SLE and healthy controls. In contrast, the truncated 35 kDa isoform of GPSM2 was significantly more highly expressed in CD4$^+$ T cells from patients with SLE as compared with healthy subjects. Antibody-mediated blockade of the 35 kDa GPSM2 isoform reduced the in vitro capacity of CD4$^+$ T cells to migrate towards the chemokine CCL20.

Conclusions A truncated GPSM2 isoform containing the GoLoco motif region is upregulated in CD4$^+$ T cells from patients with SLE and promotes CD4$^+$ T-cell migration. Targeting this isoform with specific antibodies might be a promising approach to reduce CD4$^+$ T-cell infiltration into inflamed tissues and to prevent organ damage in SLE.

Key messages

What is already known on this topic

⇒ CD4$^+$ T-cell infiltration into organs contributes to organ damage in SLE. The mechanisms underlying enhanced T-cell migration in SLE are not fully understood.

What this study adds

⇒ A truncated 35 kDa isoform of G-protein signalling modulator 2 (GPSM2) containing the GoLoco motif region is overexpressed in CD4$^+$ T cells from patients with SLE and facilitates CD4$^+$ T-cell migration.

How this study might affect research, practice or policy

⇒ Targeting the truncated 35 kDa isoform of GPSM2 might help to reduce organ infiltration by CD4$^+$ T cells in SLE.

INTRODUCTION

Organ damage induced by immune cells is a common and severe complication in SLE. CD4$^+$ T cells from patients with SLE are characterised by increased migratory functions and contribute to autoimmune inflammation and organ damage.[1 2] Recently, G-protein signalling modulator 2 (GPSM2) was identified as a promoter of CD4$^+$ T-cell migration.[3] GPSM2 is also known as Leu-Gly-Asn repeat-enriched protein (LGN) and modulates the activation of G proteins which transduce extracellular signals into integrated cellular responses.[4] The N-terminal part of GPSM2 contains 10 copies of LGN repeats, and the C-terminal part consists of four GoLoco motifs. These GoLoco motifs are short polypeptide sequences that bind to specific Gα subunits, thereby regulating the duration of intracellular signalling and the function of GPSM2.[4–6] Several isoforms of GPSM2 have been identified in human subjects, including a 35 kDa protein which is a truncated version of GPSM2 containing the four GoLoco motifs but lacking the N-terminal region with the LGN repeats.[6 7] So far, the potential role of this isoform is unknown. In this study, we aimed to reveal possible alterations in the expression of full-length GPSM2 and of the truncated 35 kDa isoform of GPSM2 which could affect the migratory capacity of CD4$^+$ T cells in SLE.

MATERIALS AND METHODS

Patients and public involvement

Patients with SLE treated at the University Hospital Cologne were able to voluntarily contribute to this research project by providing blood samples.

Samples and CD4$^+$ T-cell isolation

Peripheral blood (20 mL) was drawn from patients with SLE and from healthy individuals in the outpatient clinic of the University

Hospital Cologne. All patients with SLE fulfilled the 2019 EULAR/ACR classification criteria.[8] None of the patients reported hearing loss. Peripheral blood mononuclear cells (PBMCs) from patients with SLE and from healthy subjects were isolated using density gradient centrifugation (Pan Biotech, Aidenbach, Germany). In average, about 4×10^7 PBMCs were obtained from each donor (from about 20 mL of whole blood). $CD4^+$ T cells were isolated using MACS T-cell isolation kit (Miltenyi Biotech, Bergisch-Gladbach, Germany). Cell numbers were assessed using the CellCountess (Life Technologies GmbH, Darmstadt, Germany). $CD4^+$ T cells were purified by negative selection using the $CD4^+$ T-cell isolation kit using the QuadroMACS device (all Miltenyi Biotec). Purity of the $CD4^+$ cell population was verified by flow cytometry and was at least 96%. Viable cells were counted using the automated cell counter CellCountess (Life Technologies GmbH). In average, about 5×10^6 $CD4^+$ T cells were obtained from each donor. No significant difference in $CD4^+$ T-cell numbers was observed between healthy individuals and patients with SLE, but we obtained lower numbers of $CD4^+$ T cells from a few patients with SLE, including one patient with only 1×10^6 $CD4^+$ T cells.

Western blot analysis

Purified human $CD4^+$ cells were lysed with cell lysis buffer (BioLegend, San Diego, USA), and protein concentration was detected with the BCA Protein Assay Kit (Cell Signalling Technology, Danvers, USA). Lysates were run on 4%–15% gradient polyacrylamide gels (Bio-Rad Laboratories, Munich, Germany). Blotting was performed with the TransBlot Turbo Transfer System (Bio-Rad Laboratories). Protein (25 µg) was loaded per well. Proteins were detected with the following antibodies: HRP murine anti-human β2-Actin antibodies (Abcam, Cambridge, UK), rabbit anti-human GPSM2 antibodies (Thermo Fisher Scientific, Waltham, USA; cat. no. PA5-52555), and anti-rabbit IgG HRP-linked antibodies (Cell Signaling Technology). GPSM2 overexpressing lysate served as a positive control (Novus Biologicals, Centennial, USA; cat. no. NBL1-11310). Detection was performed by ImageJ software (NIH, USA).

Migration assay

$CD4^+$ T cells were cultured for 48 hours and stimulated with plate-bound anti-CD3 (5 µg/mL) and soluble anti-CD28 (1 µg/mL) antibodies. After 48 hours, cells were treated with blocking antibodies binding to the truncated GPSM2 isoform or control antibodies (Thermo Fisher Scientific; cat. no. PA5-115322 and MA5-16384, respectively). To study the specific function of the 35 kDa GPSM2 isoform and neutralise effects of full-length GPSM2, full-length GPSM2 was blocked with rabbit anti-human polyclonal antibodies recognising an N-terminal region of GPSM2 (amino acids 380–490) which do not bind to the 35 kDa isoform (Novus Biologicals, Centennial; cat. no. NBP3-05001). Cells (7.5×10^4) were seeded to 6.5 mm transwell with 5 µM pore size (Corning Costar, New York, USA) under cell culture conditions (5% CO_2, 37°C) for 4 hour. CCL20 (BioLegend) was used as a chemoattractant. Migrated cells were counted for 60 s using the Gallios 10/3 flow cytometer (Beckman Coulter, Krefeld, Germany).

Statistical analysis

Statistical analysis was performed using SPSS V.28. Where indicated, data were analysed by unpaired or paired Student's t-test and are presented as the mean±SEM. A p value of <0.05 was considered statistically significant.

RESULTS

Truncated 35 kDa isoform of GPSM2 is upregulated in $CD4^+$ T cells from SLE

We used western blot analysis to determine the expression levels of GPSM2 and its truncated 35 kDa isoform in $CD4^+$ T cells from healthy individuals and from patients with SLE. The patients' characteristics are summarised in table 1. Our analysis revealed no difference between full-length GPSM2 expression levels in $CD4^+$ T cells from patients with SLE and healthy individuals. The relative expression of full-length GPSM2 was 10.571±1.543 in SLE compared with 9.249±1.821 in healthy controls (p=0.5845) (figure 1A,B). In contrast, we found an almost 10 times increased expression of the truncated 35 kDa isoform of GPSM2 containing the GoLoco motif region in $CD4^+$ T cells from patients with SLE as compared with healthy subjects (2.311±0.401 in SLE vs 0.257±0.184 in healthy controls, p=0.0001) (figure 1C).

35 kDa isoform of GPSM2 is facilitates $CD4^+$ T-cell migration

Because GPSM2 is involved in $CD4^+$ T-cell migration, we studied the influence of the truncated 35 kDa isoform on the migratory function of $CD4^+$ T cells from patients with SLE. $CD4^+$ T cells were activated for 48 hours with anti-CD3 and anti-CD28 antibodies, and their capacity to migrate towards the chemokine CCL-20 was studied in an in vitro migration assay (figure 1D). Migration of $CD4^+$ T cells was significantly inhibited by antibodies binding to the truncated 35 kDa isoform of GPSM2 as compared with healthy controls (1185±190 migrated cells vs 2094±227 migrated cells in the control group, p=0.0073) (figure 1E).

DISCUSSION

To our knowledge, this is the first report showing that a truncated isoform of GPSM2 containing the GoLoco motif region is significantly upregulated in $CD4^+$ T cells from patients with an autoimmune disease. We have previously shown that full-length GPSM2 is downregulated in $CD4^+$ T cells from patients with active rheumatoid arthritis as compared with healthy individuals.[3] In contrast, our recent results revealed no difference in full-length GPSM2 expression between patients with SLE and healthy subjects. However, we found a significantly higher expression of the truncated 35 kDa isoform of GPSM2 containing the GoLoco motif region in $CD4^+$ T cells from

Table 1 Patients' characteristics

Characteristics of patients with SLE (n=10)	Normal range	
Sex (male/female)	2/8	
Age (years)	38.6 (21–68)	
Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) score	12 (8–24)	
Anti-dsDNA antibodies (kU/L)	327 (42–1286)	<100
C3 (g/L)	0.65 (0.42–1.9)	0.9–1.8
C4 (g/L)	0.08 (0.07–0.3)	0.1–0.4
Treatment (number of patients)		
Hydroxychloroquine	8	
Prednisolone	5	
Azathioprine	2	
Organ involvement		
Fever (>38.3°C)	1	
Delirium	0	
Psychosis	1	
Seizure	0	
Non-scarring alopecia	3	
Oral ulcers	2	
Subacute cutaneous or discoid lupus	7	
Acute cutaneous lupus	2	
Pleural or pericardial effusion	2	
Acute pericarditis	0	
Joint involvement	5	
Proteinuria (>0.5 g/24 hours)	2	
Lupus nephritis	1	
Laboratory parameter		
ANA ≥1:80	10	
Leucopenia (<4.000/mm3)	3	
Thrombocytopenia (<100.000/mm^3)	2	
Autoimmune haemolysis	0	
Antiphospholipid antibodies	3	
Low C3 or low C4	6	
Low C3 and low C4	3	
Anti-dsDNA or anti-Sm antibodies	10	
Characteristics of healthy controls (n=9)		
Sex (male/female)	2/9	
Age (years)	31.6 (24–62)	

Continued

patients with SLE as compared with healthy individuals.

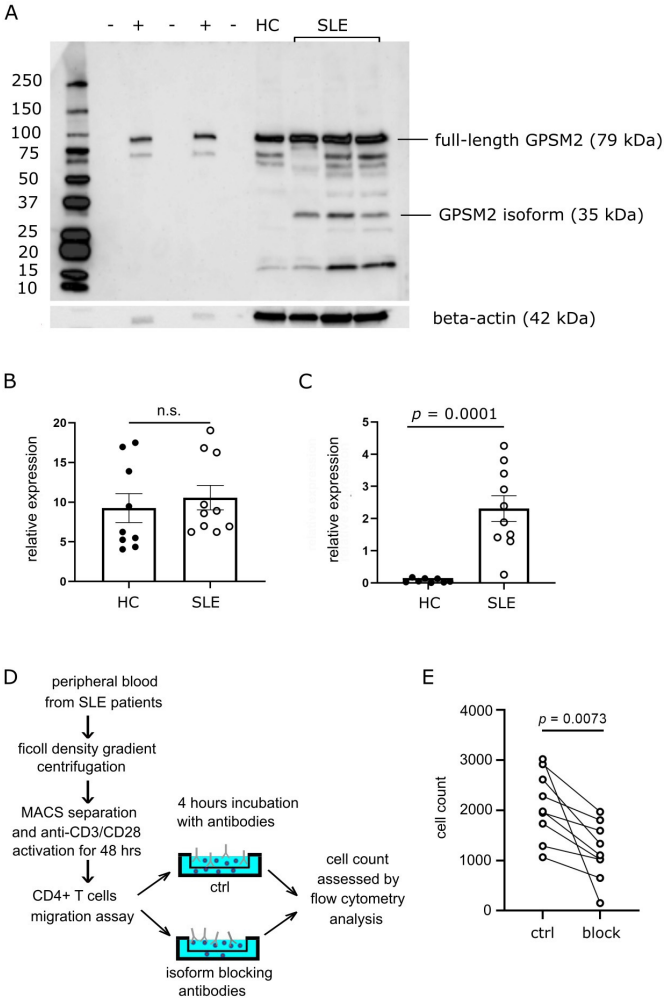

Figure 1 A truncated isoform of GPSM2 promotes CD4$^+$ T-cell migration in SLE. (A) Representative example of western blot analysis of GPSM2 and the truncated 35 kDa GPSM2 isoform in CD4$^+$ T cells from patients with SLE (SLE) and HCs. GPSM2 overexpressing lysate served as a positive ctrl (+); no protein served as a negative ctrl (–). (B) Comparison of GPSM2 expression in CD4$^+$ cells from patients with SLE (n=10) and healthy individuals (n=9). (C) Expression of the truncated GPSM2 isoform in CD4$^+$ cells from patients with SLE (n=10) and healthy individuals (n=9). (D) Schematic view of the experimental set-up of an in vitro T-cell migration assay. (E) Inhibition of the truncated GPSM2 isoform with blocking antibodies (block) or ctrl antibodies leads to decreased migration of CD4+ T cells from patients with SLE (n=9). Data are presented as mean±SEM. Significance was calculated using unpaired (B,C) or paired (E) Student's t-test. ctrl, control; GPSM2, G-protein signalling modulator 2; HC, healthy control; n.s., not significant.

Moreover, our data indicate that the truncated 35 kDa isoform of GPSM2 is implicated in CD4+ T-cell migration in SLE.

We used CCL20 as a chemoattractant in the in vitro migration assay because upregulation of GPSM2 has been linked to elevated CCL20 expression.[9] In addition, the CCL20 receptor CCR6 is abundantly expressed on Th17 cells, T helper 22 (Th22) cells, Treg cells and a small percentage of follicular T-helper cells. Growing evidence suggests that these cell types are involved in the pathogenesis of SLE, and it has been shown that the amount of kidney-infiltrating Th17 cells is associated with clinical parameters like urine protein level in glomeruli, serum creatinine and creatinine clearance in active lupus nephritis.[10–12] Moreover, elevated levels of CCR6+ Th22 cells correlate with skin and renal impairment in SLE.[13] Enhanced migration of CCR6+CD4+ T cells towards CCL20 due to elevated expression of the 35 kDa isoform might therefore represent a possible pathogenic mechanism of immune infiltration and organ damage in SLE.

Truncated isoforms of GPSM2 have been recently identified in human subjects and have been linked to hearing loss.[7] Interestingly, hearing loss occurs significantly more often in patients with SLE as compared with healthy individuals.[14] Here we show that the truncated isoform of GPSM2 containing the GoLoco motif region promotes CD4+ T-cell migration and is overexpressed in patients with SLE. This mechanism may explain the enhanced migratory capacity of CD4+ T cells in SLE. Targeting of the truncated GPSM2 isoform with specific antibodies could therefore represent a possible strategy to inhibit CD4+ T-cell infiltration into inflamed tissues and to reduce organ damage in SLE.

Contributors RLE, CB, JT, VG-N, AM, ES-K and SY performed the experiments. RLE, CtP, DS, JS and DMK analysed the data. All authors contributed to the discussion of results. DMK drafted the manuscript. All authors contributed to, read and approved the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the ethics committee of the University Hospital Cologne (number 13-091). The participants gave informed consent to participate in the study before taking part.

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