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ORIGINAL ARTICLE

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Overcoming treatment unresponsiveness mediated by P-glycoprotein overexpression on lymphocytes in refractory active systemic lupus erythematosus

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Abstract P-glycoprotein (P-gp) expels various drugs from cells, resulting in multidrug resistance, including against glucocorticoids. Here, we present a case of systemic lupus erythematosus (SLE) that suggests the importance of initial intensive treatment in overcoming unresponsiveness due to P-gp overexpression on activated lymphocytes. A 28-year-old woman had been diagnosed with highly active SLE including severe pericarditis, hemolytic anemia, lupus nephritis, and retinopathy. The disease activity of SLE progressed despite 1 mg/kg per day oral prednisolone. At the time, P-gp expression was extremely high, as evaluated by flow cytometric analysis on peripheral lymphocytes. After intensive treatment with three courses of methylprednisolone pulse therapy and plasmapheresis, we succeeded in controlling disease activity in association with marked reduction of P-gp overexpression; namely, the clinical symptoms immediately improved along with the reduction of P-gp expression. These results imply that patients with highly active SLE might have drug unresponsiveness that is mediated by P-gp overexpression on lymphocytes. Therefore, downregulation of P-gp by initial intensive immunosuppressive therapy might be important for overcoming glucocorticoid resistance. We also propose that measurement of P-gp on lymphocytes is a useful test for prediction of drug resistance and may assist in the selection of appropriate initial treatment.

Key words Lymphocyte · P-glycoprotein (P-gp) · Systemic lupus erythematosus (SLE) · Treatment unresponsiveness

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Introduction

Glucocorticoids and immunosuppressive agents are essential for the control of disease activity in the management of autoimmune disorders with activated autoreactive lymphocytes. However, patients often do not respond to initial treatment with glucocorticoids, and such unresponsiveness to drugs is one of the most important issues to be overcome in the treatment of systemic autoimmune diseases. Among the multiple mechanisms of multidrug resistance, overexpression of P-glycoprotein (P-gp), a 170-kDa product of the multidrug resistance-1 gene, has emerged as the major molecule involved in multidrug resistance during chemotherapy for various malignancies.[1–4] P-glycoprotein is a member of the ATP-binding cassette transporter superfamily of genes and functions as an energy-dependent transmembrane efflux pump. Overexpression of P-gp results in reduction of intracellular concentrations of various drugs, including glucocorticoids.[5–8]

Resistance to chemotherapy induced by P-gp is closely associated with the prognosis of human malignancies.[1–4] Treatment resistance is also common in patients with nonmalignant conditions, such as systemic autoimmune diseases like systemic lupus erythematosus (SLE).

The present study was designed to determine the role of P-gp overexpression in resistance to glucocorticoid therapy in SLE. For this purpose, we investigated the relationship between P-gp expression on lymphocytes and the clinical response to treatment in a patient with highly active SLE.

Materials and methods

Isolation of peripheral blood mononuclear cells

We isolated peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation using Lymphocyte Separation Medium 50494 (Pharmacia Biotech, Uppsala, Sweden) as described previously.[9,10] Peripheral blood mononuclear cells were suspended in phosphate-buffered

saline (PBS) during all experimental procedures and used immediately. The study was approved by the Human Subject Research Committee of the University of Occupational and Environmental Health, School of Medicine, and informed consent was obtained from the patients and healthy donors.

Flow cytometric analysis

Staining and flow cytometric analysis of PBMCs were conducted by standard procedures as described previously using a FACScan (Becton Dickinson, Mountain View, CA, USA).[9,10] Briefly, PBMCs (2×10^5 cells/well) were initially incubated with polyclonal γ-globulin (10 μg/ml, Mitsubishi Welpharma, Osaka, Japan) for the blocking of Fc-receptors, and then incubated with MRK-16, a specific monoclonal antibody (mAb) against P-gp,[11] followed by fluorescein isothiocyanate-conjugated antimouse IgG Ab (Fujisawa, Osaka) in FACS medium consisting of PBS, 0.5% human serum albumin (HSA; Mitsubishi Welpharma), and 0.2% NaN_3 (Sigma Aldrich, Tokyo, Japan). For the two-color analysis, we incubated PBMCs with phycoerythrin (PE)-conjugated CD4 mAb, CD8 mAb, or CD19 mAb (Fujisawa) after blocking free antimouse IgG-binding sites with irrelevant antibodies. Monoclonal antibody-two-color-stained cells were detected by electronic gating based on their CD4, CD8, or CD19 expression using a fluorescence-activated cell sorter (FACScan). Amplification of mAb binding was provided by a three-decade logarithmic amplifier. Quantification of the cell surface antigens on one cell was performed using QIFIKIT beads (Dako, Kyoto, Japan) as reported previously.[12]

Case report and results

A 28-year-old woman was admitted to our university hospital and SLE was diagnosed in 2002, based on the criteria of the American College of Rheumatology (ACR),[13] including the presence of marked pericarditis (Fig. 1), persistent proteinuria, pancytopenia, and serological abnormalities: positive antinuclear antibody, positive anti-double-stranded (ds) DNA antibody (Table 1). The SLEDAI (SLE disease activity index) score[14] was 27 and disease activity was considered extremely high. She also developed life-threatening symptoms such as marked pericarditis, hemolytic anemia, severe retinopathy with cytoid bodies, and bleeding. Renal biopsy confirmed the diagnosis of International Society of Nephrology/Renal Pathology Society (ISN/RPS) type III (A) lupus nephritis.[15]

As shown in Fig. 2, initial treatment with 1 mg/kg per day of prednisolone (PSL) completely failed to suppress disease activity, and the hemolytic anemia and hypertension became more severe. Although two courses of combination therapy with methyl-PSL pulse therapy (1 g/day for 3 days) and plasmapheresis were employed, hypertension, hypocomplementemia, and pericarditis did not improve. After the third methyl-PSL pulse treatment, which was administered 3 weeks after admission, marked improvement was immediately noted in clinical features, including hemolytic anemia, pericarditis, nephritis, and hypocomplementemia, and the laboratory data finally returned to normal levels over the following 2 weeks (Figs. 2 and 3A,B).

To investigate the association between P-gp expression on peripheral lymphocytes and unresponsiveness to corticosteroid as shown in our patient, we performed two-color analysis using anti-CD8, -CD4, and -CD19 antibodies and then determined P-gp expression on each subset of lymphocytes by flow cytometric analysis on admission, at 3 weeks, and at 5 weeks after admission. On admission, P-gp expression was extremely high on peripheral lymphocytes from each subset of lymphocytes (Fig. 4) in comparison with healthy donors (Table 2). Two courses of combination therapy with methyl-PSL pulse therapy and plasmapheresis resulted in marked reduction of the number of P-gp molecules expressed per cell (molecules/cell) as well as a substantial decrease in the percentage of P-gp positive cells (% positivity) in each lymphocyte subset. Finally, P-gp-positive lymphocytes almost disappeared by 5 weeks after admission in parallel with clinical resolution of SLE.

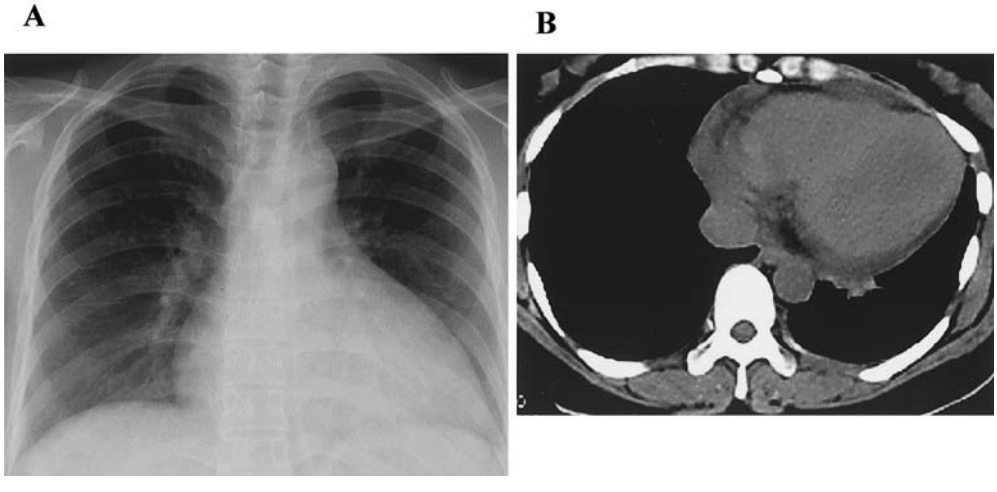

Fig. 1A,B. Chest X-ray and computed tomography findings on admission. **A** Chest X-ray; cardiothoracic ratio 67%. **B** Chest computed tomography revealed marked pericardial effusion due to pericarditis

Table 1. Laboratory findings on admission

Urine (400 ml/day)		Serology	
Protein	(+3) 0.5 g/day	Albumin	3.2 g/dl
Sugar	(−)	ALT	25 IU/l
Occult blood	(+3)	AST	19 IU/l
Cast	(−)	LDH	358 IU/l
		Creatinine	0.6 mg/dl
CBC		CRP	2.6 mg/dl
Leukocytes	3100/μl	IgG	2022 mg/dl
Lymphocytes	11%	IgA	184 mg/dl
Red blood cells	293×10^4/μl	IgM	169 mg/dl
Hemoglobin	8.2 g/dl	C3	24 mg/dl
Reticulocytes	18%	C4	<5 mg/dl
Platelets	13.8×10^4/μl	CH_{50}	<5 U/ml
		ANA	5120×
ESR	120 mm/h	Anti-dsDNA Ab	>400 IU/ml
Arterial blood gases		Coombs test	
PaO_2	77 mmHg	Indirect	(−)
$PaCO_2$	33.7 mmHg	Direct	(++)

ESR, erythrocyte sedimentation rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; CRP, C-reactive protein; CH_{50}, 50% hemolytic complement activity; ANA, antinuclear antibodies

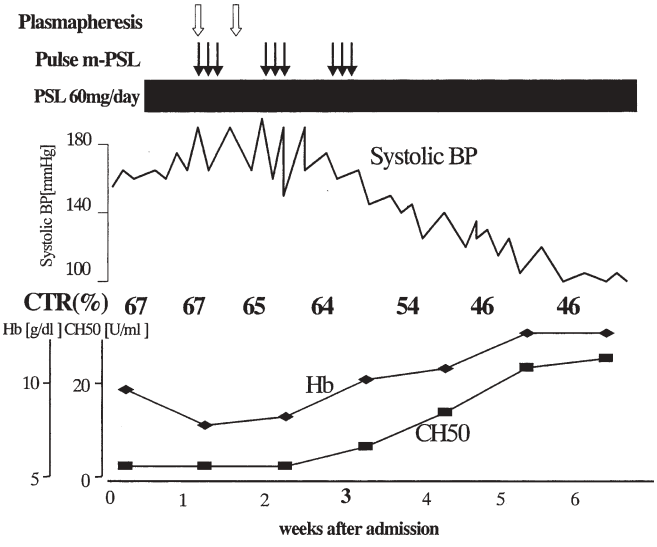

Fig. 2. Clinical course. *PSL*, prednisolone; *m-PSL*, methylprednisolone; *BP*, blood pressure; *CTR*, cardiothoracic ratio; *Hb*, hemoglobin; *CH50*, 50% hemolytic complement activity

Discussion

Glucocorticoids are key drugs for the treatment of patients with active SLE.[16,17] Glucocorticoids circulate in the blood in free forms or as complexes with corticosteroid-binding globulin (CBG), which is also called transcortin, or as albumin-binding forms. The drugs then shift into target cells and exert their biological actions.[18] Although most patients with SLE respond to glucocorticoid treatment, some show a poor initial response. Several mechanisms could explain glucocorticoid unresponsiveness. Patients with SLE often develop peritonitis or hypoalbuminemia, which induces glucocorticoid malabsorption from the intestine.[19,20] Rapid glucocorticoid degradation is thought to be another reason for the poor therapeutic response.[21] However, these problems can be rapidly overcome by intravenous infusion of high-dose glucocorticoids. Although our patient had hypoalbuminemia and serositis, initial intravenous injection of methyl-PSL did not improve the disease activity at all.

Extremely high expression of P-gp was evident on lymphocytes in our patient. We found that expression of P-gp on lymphocytes induced by activation stimuli such as interleukin-2, a potent stimulator of lymphocytes,[22,23] increased in serum of patients with active SLE[24–26] in a dose-dependent manner.[27] In murine peripheral $CD4^+$ cells, only an "activated/memory" phenotype of $CD4^+$ cells exhibits P-gp activity.[28] Moreover, the positivity of CD69, an early T-cell activation maker, on CD3 cells in SLE patients closely correlated with SLEDAI.[29] Therefore in our patient, extremely high expression of P-gp on lymphocytes was induced by lymphocyte activation according to high disease activity on admission.

P-glycoprotein is known to expel intracellular drugs, including glucocorticoids;[5–8] we also found that P-gp expression on activated lymphocytes resulted in a marked decrease of intracellular glucocorticoid concentration in vitro.[27] Others have reported that decreased cytoplasmic glucocorticoid concentration as a result of increased P-gp-mediated efflux of glucocorticoid from lymphocytes is one of the mechanisms of glucocorticoid resistance in inflammatory bowel disease and asthma.[30,31] We therefore considered one of the causes of the poor response to glucocorticoid to be the decrease of intracellular glucocorticoid concentration due to expulsion through P-gp. Two courses of intensive treatment with methyl-PSL pulse therapy markedly reduced P-gp expression. Drug resistance mediated through P-gp was apparently abolished by the initial two courses of methyl-PSL pulse therapy. Finally, the disappearance of P-gp led to a successful reduction of disease

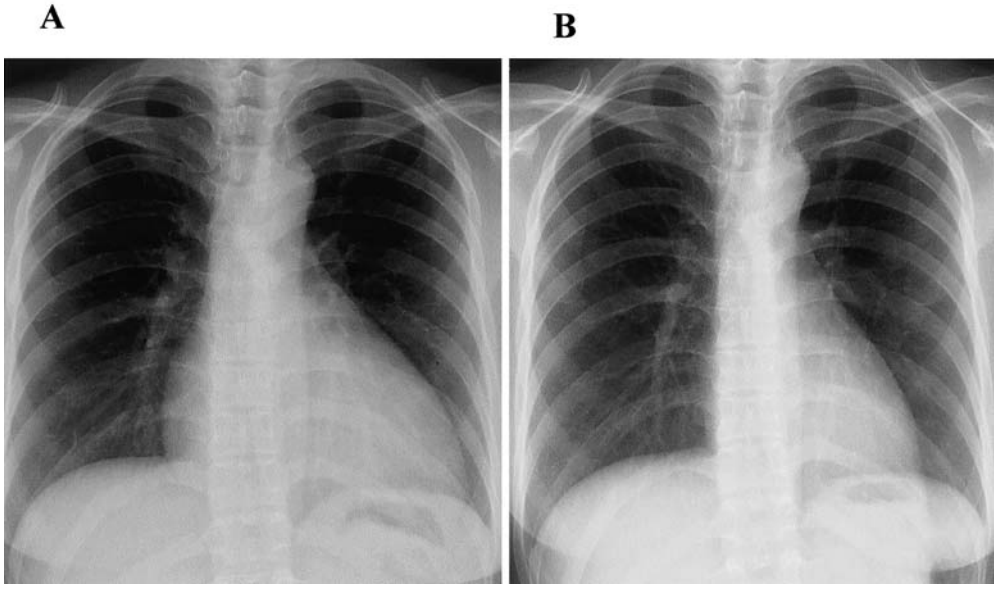

Fig. 3A,B. Chest X-ray after intensive immunosuppressive therapy. **A** Three weeks after admission; cardiothoracic ratio (CTR) 64% 5 days after two courses of methylprednisolone (m-PSL) pulse therapy and plasmapheresis. **B** Five weeks after admission; CTR 46% 10 days after three courses of methyl-PSL pulse therapy

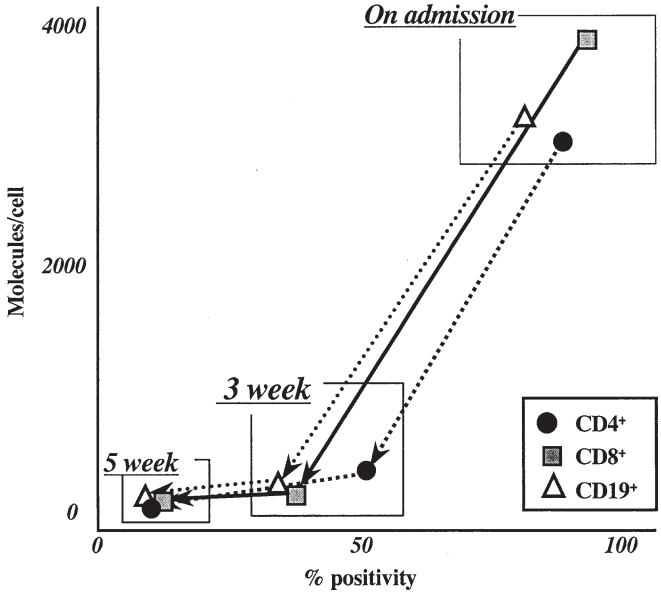

Fig. 4. Reduction of P-glycoprotein (P-gp) expression caused by treatment. Flow cytometric analysis showed P-gp on peripheral *CD4*$^+$ (*circles*), *CD8*$^+$ (*squares*), and *CD19*$^+$ (*triangles*) cells after admission. *Ordinate*: number of molecules expressed per cell, calculated using standard QIFIKIT beads; *abscissa*: percent binding of anti-P-gp monoclonal antibody, MRK16, calculated using a three-decade logarithmic amplifier

activity after the third course of methyl-PSL pulse therapy and subsequent treatment with oral PSL.

In conclusion, we propose that SLE patients with highly active disease could initially develop drug resistance, including poor responsiveness to several immunosuppressants and glucocorticoids, caused by P-gp overexpression on lymphocytes. In such cases, suppression of P-gp expression by the intensive immunosuppressive treatment is important to overcome glucocorticoid resistance. In addition, we propose that measurement of P-gp on lymphocytes is useful for the prediction of glucocorticoid resistance and as an indicator of drug resistance in general.

Table 2. P-Glycoprotein expression on normal lymphocytes (n = 10)

Subset of lymphocyte	P-Glycoprotein expression	
	% Positivity	Molecules/cell
$CD4^+$ cell	4.9 ± 2.1	66.7 ± 35.7
$CD8^+$ cell	6.0 ± 4.1	71.0 ± 47.9
$CD19^+$ cell	3.1 ± 3.1	206.5 ± 119.9

Flow cytometric analysis showed P-glycoprotein on peripheral $CD4^+$, $CD8^+$, and $CD19^+$ cells of healthy donors. Data represent mean ± SD of ten independent experiments
% positivity, percent binding of anti-P-glycoprotein monoclonal antibody, MRK16, calculated using a three-decade logarithmic amplifier; molecules/cell, number of molecules expressed per cell, calculated using standard QIFIKIT beads

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