

## Review

# Relevance of multidrug resistance 1 and P-glycoprotein to drug resistance in patients with systemic lupus erythematosus

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**Summary.** Although corticosteroids and immunosuppressants are widely used for the treatments of various autoimmune diseases such as systemic lupus erythematosus (SLE), we often experience patients with SLE who are resistant to these treatments. P-glycoprotein (P-gp) of membrane transporters, a product of the multiple drug resistance (MDR)-1 gene, is known to play a pivotal role in the acquisition of drug resistance to chemotherapies in malignancy. However, the relevance of MDR-1 and P-gp to resting and activated lymphocyte, major targets of the treatments in autoimmune diseases, remains unclear. We found that peripheral lymphocytes in patients with SLE express P-gp on the surface and its expression is highly correlated with disease activity. P-gp on lymphocytes is induced by not only genotoxic stresses but also activation stimuli such as cytokines, resulting in active efflux of corticosteroids from cytoplasm of lymphocytes, resulting in drug-resistance and high disease activity. However, the addition of P-gp antagonists such as ciclosporin A and inhibitors of P-gp synthesis successfully reduce efflux of corticosteroids from lymphocytes *in vitro* and these results imply that P-gp antagonists and P-gp synthesis inhibitors could work in order to overcome drug-resistance *in vivo*. Therefore, we propose that the measurement of P-gp on lymphocytes is a useful marker to indicate drug resistance and requirement of antagonists and/or intensive treatments to overcome drug resistance in active SLE patients.

**Key words:** Multidrug resistance 1 gene, P-glycoprotein, Lymphocytes, Systemic lupus erythematosus, Autoimmune disease

## Introduction

Systemic lupus erythematosus (SLE) and rheumatoid arthritis are representative autoimmune diseases in which autoreactive T cells and B cells play a pivotal role in the pathological processes and immune complexes consisting of antigens and autoantibodies secreted from activated B cells cause severe damages of various tissues and organs. Although corticosteroids and immunosuppressants are widely used for treatments, we often experience patients with SLE who are refractory to these conventional treatments, particularly, failure to control high disease activity, resulting in life threatening (Jorgensen and Maillefert, 2000). Therefore, drug resistance of lymphocytes is one of the important subjects to be overcome with regard to the treatment of SLE. Among the multiple mechanisms of multidrug resistance, this review documents the relevance of P-glycoprotein (P-gp) encoded by the multidrug resistance-1 (MDR-1) gene on lymphocytes to drug resistance in SLE.

## The mechanisms of drug resistance

Overexpression of P-gp, a 170-kDa product of the MDR-1 gene, has emerged as a major molecule involved in multidrug resistance during chemotherapy for various malignancies (Leonard et al., 2002; Kuwano et al., 2003; McKeegan et al., 2003). P-gp is a member of ATP-binding cassette transporter superfamily and functions as an energy-dependent transmembrane efflux pump. P-gp is recognized by structurally diverse, hydrophobic/amphiphilic substrates, ranged 300-2000 Da, catches these substrates like a "vacuum cleaner" when they pass through the cell membrane, and pumps them out of the cells at the expense of ATP hydrolysis. Therefore, overexpression of P-gp results in reduction of intracellular concentrations of xenobiotics, drugs and

poisons, such as vinca alkaloids, anthracyclines, verapamil, some immunosuppressants and corticosteroids (Fisher et al., 1996; Meijer et al., 1998).

### The regulatory mechanisms of the MDR-1 gene in lymphocytes

We and others have reported that transcription of MDR-1 is directly regulated by human Y-box-binding protein-1 (YB-1), a MDR-1 transcription factor, and that activation of YB-1 in various tumor cells is induced in response to genotoxic stresses (Ohga et al., 1998) such as ultraviolet light (Uchiumi et al., 1993), anticancer agents (Kohno et al., 1989), serum starvation (Tanimura et al., 1992), heat shock (Miyazaki et al., 1992) and multiple drugs, including vinca alkaloids and corticosteroids (Chaudhary and Roninson, 1993). However, the relevance of MDR-1 and P-gp to resting and activated lymphocyte remains unclear.

We have explored that MDR-1 transcription in lymphocyte is also induced by not only genotoxic stresses but also activation stimuli such as IL-2, a potent stimulator of lymphocytes (Tsujimura et al., 2004), based on the following sequence of events; activation and translocation of YB-1 by IL-2, transcription of MDR-1 by the binding of the activated YB-1, expression of P-gp on the cell surface, and excretion of the intracellular dexamethasone added *in vitro* (Fig. 1). Furthermore, we found that the expression of P-gp on lymphocytes via activation of YB-1 was induced by adhesion with the extracellular matrix involving hyaluronan, and inflammatory cytokines, such as IL-6 and TNF- $\alpha$ . Therefore, the overexpression of P-gp on lymphocyte in accordance with lymphocyte activation results in the development of multi-drug resistance.

### Clinical relevance of P-gp expression on lymphocytes to drug resistance in SLE

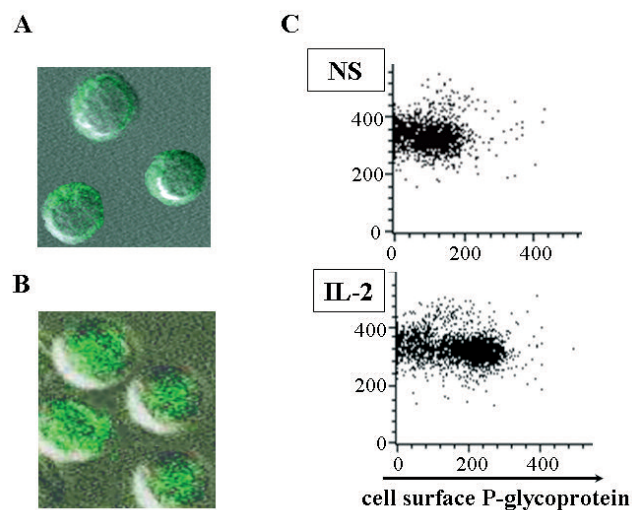
For the sake of treatments of SLE, although lymphocytes are major targets of the treatments by corticosteroids and immunosuppressant and we often experience drug resistant SLE patients with active disease activity, the relevance of MDR-1 and P-gp to resting and activated lymphocytes in these patients remains unclear. We assessed the expression of P-gp using mAb against MRK-16 epitope of P-gp on peripheral lymphocytes in 80 SLE patients and 20 normal volunteers. P-gp was highly expressed on most of the peripheral CD4+, CD8+, and CD19+ lymphocytes in SLE patients, the amounts of P-gp on lymphocytes in SLE showed a variety of levels, whereas normal lymphocytes revealed only marginal expression.

It is noteworthy that the expression levels of P-gp on SLE lymphocytes highly correlated with the disease activity of each patient estimated by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Tsujimura et al., 2005b). Furthermore, we clarified that

corticosteroid-unresponsiveness is closely associated with the level of P-gp expression on lymphocytes in active SLE patients. We divided patients with SLE into two groups; the first group "low responders", represented patients whose SLEDAI score was more than 12 points, despite taking more than 0.5 mg/kg/day of prednisolone (PSL), and the second group "responders", who responded well with less than 1.0 mg/kg/day of PSL equivalent. The level of P-gp on the CD4+, CD8+, and CD19+ lymphocytes was markedly increased in the "low responders".

In order to investigate the association between expression of P-gp and exclusion of drugs through P-gp *in vitro*, intracellular and extracellular concentration of dexamethasone was evaluated by C/M ratio, an index of intracellular dexamethasone concentration (C) and extracellular dexamethasone in conditioned medium (M) ratio. By the *in vitro* analyses of peripheral lymphocyte of SLE patients, intracellular dexamethasone in lymphocytes was significantly decreased, in accordance with an enhancement of P-gp expression on lymphocytes, which reached much lower levels, compared with normal volunteers. Furthermore, the intracellular dexamethasone levels decreased significantly in lymphocytes of these "low responders" compared with "responders" and normal volunteers (Tsujimura et al., 2005b).

Therefore, the high expression of P-gp on lymphocytes in accordance with lymphocyte activation by various stimuli might lead to active efflux of



**Fig. 1.** IL-2 induces nuclear translocation of YB-1 and cell surface P-glycoprotein expression. A and B, Immunostaining and confocal microscopy analysis of YB-1 in resting lymphocytes (A) and IL-2-activated lymphocytes (B). Magnification, x 600. C, Flow cytometry and subsequent dot plot analyses; X-axis is P-glycoprotein and Y-axis is CD4 of lymphocytes without stimulation (NS) or stimulated with IL-2 (IL-2).

## Drug resistance in SLE

corticosteroids from the cytoplasm, resulting in the development of drug resistance and failure to control disease activity in SLE patients with high disease activity (Fig. 2).

### Overcoming drug unresponsiveness mediated by P-gp in active SLE

P-gp expression has been shown to correlate negatively with survival in some solid tumors and various hematological malignancies. Several non-cytotoxic drugs (e.g. calcium channel blockers, quinolines, antibiotics, calcineurin inhibitors) are also putative substrates of P-gp, and some have been shown to inhibit drug efflux competitively (Fisher et al., 1996). In fact, in chemotherapy of malignancies, several clinical trials of competitive P-gp antagonists examined their effects on the overcoming of multidrug resistance induced by P-gp (Advani et al., 1999; List et al., 2002).

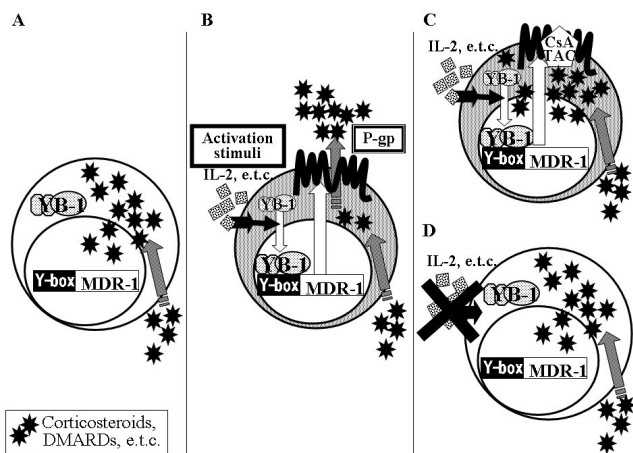
Ciclosporin A and tacrolimus are known to be a calcineurin inhibitor, inhibiting NF-AT-dependent IL-2 transcription in lymphocytes, but they are also competitive antagonists of P-gp. We have demonstrated that excretion of dexamethasone in activated lymphocytes inhibited by ciclosporin A and tacrolimus in a concentration-dependent manner *in vitro*. Furthermore, ciclosporin A and tacrolimus reduced the excretion of dexamethasone in IL-2-activated

lymphocytes at the lower concentration than clinical trough levels used as a calcineurin-inhibitor (Tsujimura et al., 2004). Actually, the excretion of dexamethasone *in vitro* in lymphocytes of “low responders” SLE patients was efficiently inhibited by ciclosporin A (Tsujimura et al., 2005b). Therefore, we propose that the treatment with P-gp antagonists such as ciclosporin A and tacrolimus could be useful for highly active SLE patients who do not respond to corticosteroids.

Otherwise, we provided intensive immunosuppressive treatments in addition to corticosteroids to 10 patients with “low responders” SLE. All 10 patients had high disease activity with a SLEDAI score of more than 12 points, despite taking more than 0.5 mg/kg/day of PSL and followed by additional intensive immunosuppressive treatments of intravenous infusion of cyclophosphamide, plasmapheresis, ciclosporin A or methyl-prednisolone pulse therapy. These intensive immunosuppressive treatments successfully controlled disease activity, in accord with a marked reduction of P-gp on the lymphocytes in SLE patients (Tsujimura et al., 2005a,b). Therefore, we propose that down-regulation of P-gp by the intensive immunosuppressive therapy also might be important for overcoming corticosteroid-resistance (Fig. 2).

### Conclusion

In SLE patients with highly active disease, activated lymphocytes by various stimuli such as cytokines could result in major cause of drug resistance by the following sequence of events; activation of YB-1, translocation of YB-1 into nuclei, transcription of MDR-1 through YB-1, expression of P-gp on the cell membrane, excess efflux of intracellular multi-drugs including corticosteroids and immunosuppressants, resistant to these treatments and high disease activity. Actually, we found that peripheral lymphocytes in patients with SLE express P-gp on the surface and its expression is highly correlated with disease activity. However, the addition of P-gp antagonists such as ciclosporin A and tacrolimus and inhibition of P-gp synthesis by intensive treatments successfully reduce efflux of corticosteroids from lymphocytes *in vitro* and these results imply that P-gp antagonists and P-gp synthesis inhibitors could work in order to overcome drug-resistance *in vivo*. Accordingly, we propose that measurement of levels of P-gp expression on peripheral blood lymphocytes is useful for the assessment of drug resistance and is a good marker for selection of P-gp antagonists such as ciclosporin A and tacrolimus, and for application of the intensive immunosuppressive treatments in SLE patients with highly active disease.



**Fig 2.** Schematic of relevance of P-glycoprotein to drug resistance of SLE. **A.** P-glycoprotein (P-gp) on resting lymphocytes in normal subjects and inactive SLE patients revealed only marginal expression. **B.** In SLE patients with high disease activity, various stimuli induce P-gp on lymphocytes, which lead to active efflux of drugs from lymphocytes, resulting in the drug-unresponsiveness and failure to control disease activity. **C and D.** Both inhibition of P-gp by competitive antagonists such as ciclosporin A (**C**) and reduction of P-gp achieved by intensive immunosuppressive treatments (**D**) could overcome drug-resistance induced by overexpression of P-gp in highly active SLE patients.

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