Pediatr Nephrol (2006) 21: 1274–1280
DOI 10.1007/s00467-006-0187-2

ORIGINAL ARTICLE

Anna Wasilewska · Walentyna Zoch-Zwierz · Miroslawa Pietruczuk · Grzegorz Zalewski

Expression of P-glycoprotein in lymphocytes from children with nephrotic syndrome, depending on their steroid response

Received: 15 February 2006 / Revised: 4 April 2006 / Accepted: 7 April 2006 / Published online: 8 June 2006

Abstract The aim of this study was to examine the expression of P-glycoprotein (P-gp) in CD3 lymphocytes of children with nephrotic syndrome (NS) in relation to their clinical response to glucocorticoid (GC) treatment. The examinations were performed on two groups. The study group (I) consisted of 88 children aged 2.0–20.0 years with NS, divided according to their clinical response to GC: NFR—non-frequent relapse NS; FR—frequent relapse NS; SD—steroid-dependent NS. The control group (II) consisted of 18 healthy children never treated with GC. We measured P-gp expression on CD3 lymphocytes of patients with NS using a flow cytometry assay. The CD3/P-gp was significantly higher than in controls. The difference was higher in SD (P=0.0001) and FR - (P=0.0002) group. The difference in NFR was smaller. Mean CD3/P-gp (in percent) was twice as high in SD children than in NFR, and the difference, as between FR and NFR, was statistically significant (P<0.01). Worse response to GC or dependency may be due to overexpression of P-gp. Further examinations are necessary to establish whether increased P-gp activity is a result of MDR-1 polymorphism and to determine GC response, or to ascertain if such activity is only a result of GC therapy.

Keywords Children · Glucocorticoids · Nephrotic syndrome · P-glycoprotein

A. Wasilewska (✉) · W. Zoch-Zwierz · G. Zalewski
1st Department of Pediatrics,
Medical University of Białystok,
ul. Waszyngtona 17,
15-274 Białystok, Poland
e-mail: annwasil@interia.pl
Tel.: +48-85-7450823
Fax: +48-85-7421838

M. Pietruczuk
Department of Haematological Diagnostics,
Medical University of Białystok,
Białystok, Poland

Introduction

Childhood nephrotic syndrome was once considered a benign condition because of the good response to steroid treatment in the majority of patients. Most comprehensive reviews of childhood nephrotic syndrome (NS) have shown that the response rate to steroids has been reported to be at least 80%, and even 90% [1, 2]. Despite response to steroids, over one-third of patients with steroid-sensitive NS (SSNS) relapse or develop a steroid-dependent course of disease [2, 3]. Our clinical findings suggest that, in children with good initial steroid responsiveness, sometimes during the course of diseases the response to steroid treatment becomes worse, and, sometimes, late steroid resistance is observed. Resistance to the therapeutic effects of corticosteroids is recognised in patients with asthma [4], inflammatory bowel diseases [5] and rheumatoid arthritis [6].

An acquired worse response to corticosteroids in NS may be related to drug resistance mechanisms. Among these, one of the most extensively studied is so-called multidrug resistance-1 (MDR-1), which is characterised by the overfunction of one 170 kDa P-glycoprotein (P-gp) [7]. P-gp belongs to the ATP binding cassette superfamily of transport proteins, first described by Juliano and Ling in 1976 [8]. It is richly expressed in many tissues, and, in the haemopoietic system, peripheral blood mononuclear cells, macrophages, natural killer, dendritic cells and T and B lymphocytes all express P-glycoprotein at varying levels [9].

The MDR-1 phenotype involves decreased sensitivity to several agents, including vinca alkaloids, anthracyclines and glucocorticoids [10]. It is the consequence of the drug expulsion by P-gp that diminishes the intracellular drug concentration, thus reducing its therapeutic action. However, still little is known about the possible role of P-gp in autoimmune diseases that require therapy with drugs actively effluxed by this pump, e.g. prednisone.

The aim of this study was to explore the expression of P-gp in CD3 lymphocytes in the peripheral blood of children with nephrotic syndrome in relation to their clinical response to glucocorticoid treatment.

Subjects and methods

The examinations were performed on two groups of children. The study group (I) consisted of 88 children (♀- 39, ♂- 49), aged 2.0–20.0 years (mean 10.0±4.88 years) with nephrotic syndrome. They were divided into three groups, depending on their clinical response to glucocorticoid treatment: NFR—non-frequent relapse NS; FR—frequent relapse NS; SD—steroid-dependent NS.

Definitions NS was defined as urinary protein excretion of >40 mg/m^2 per hour and hypoalbuminaemia of <25 g/dl, according to the criteria of the International Study of Kidney Disease in Children (ISKDC) [1].

Remission of NS was characterised by the disappearance of albuminuria for at least 3 consecutive days.

Frequent relapsers were defined according to the ISKDC criteria (more than two relapses in the initial 6 months after presentation or more than four per year during follow-up) [11].

Steroid dependency was defined according to the guidelines of the of Arbeitsgemeinschaft für Pädiatrische Nephrologie (at least two relapses during alternate day treatment with prednisone or within 2 weeks after cessation) [12].

All the examined children were treated with the standard initial therapy, consisting of daily administration of prednisone 60 mg/m^2 body surface area for 4 weeks, followed by 40 mg/m^2 given on alternate days, followed by various tapering on alternate days. Relapses were treated with prednisone 60 mg/m^2 administered daily until remission was achieved, followed by 40 mg/m^2 on alternate days.

In steroid-dependent patients, maintenance alternate day dosage of prednisone was instituted. The alternate dose was gradually tapered to determine each patient's individual threshold at which relapse occurred.

In children with frequent relapses, steroid dependency or serious side effect of steroids alternative treatment was used. Cyclophosphamide therapy was administered in an 8-week course in 29/88 children; Imuran (azathioprine) was used only in 6/88 children. The cytotoxic treatment was finished at least 6 months before the examinations took place. Because cyclosporine treatment may influence P-gp expression, we excluded all the children treated with this drug [13].

The children were examined in remission of NS and had not received steroids for at least 3 months in subgroups NFR and FR. In SD subgroup 7/18 children received prednisone at a dose not exceeding 0.5 mg/kg body weight (b.w.) on alternate days.

Table 1 The characteristics of the patients with NS according to their clinical steroid response (*CF* cyclophosphamide, *AZA* azathioprine, *MCNS* minimal change nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis)

Characteristic		Non-frequent relapse NS (NFR) (*n*=44) Mean ± SD; median (range)	Frequent relapse NS (FR) *n*=26	Steroid-dependent NS (SD) *n*=18
Male/female		22/20	15/11	10/8
Age of onset (years)		5.64±3.05	4.68±3.05	5.08±4.21
		5.5 (1.5–15.5)	3.5 (1.5–12.5)	2.5 (1.5–14.0)
Age at evaluation (years)		10.20±4.33	11.03±4.68	11.0±6.59
		11.0 (3.0–19.0)	12.0 (2.0–18.0)	13.5 (2.0–20.0)
Duration of follow up (years)		4.55±3.19	6.35±4.27	5.91±5.64
		3.5 (0.5–16.5)	5.75 (0.5–15.5)	4.25 (0.5–17.5)
Number of NS episodes		3.45±1.04	8.0±2.26	7.0±2.35
		3.5 (1.0–5.0)	8.0 (4.0–12.0)	7.0 (4.0–12.0)
Time to remission (days)		10.29±5.28	15.76±4.99	15.33±6.01
		8.5 (4.0–24.0)	15.5 (6.0–25.0)	17.5 (5.0–23.0)
Family history of NS		0/44	2/26	1/18
Alternative therapy		CF-3/44	CF-17/26	CF-9/18
			AZA-3/26	AZA-3/18
Total dose of prednisone mg/kg b.w.		965.68±422.13	1903.46±625.12	1667.22±990.76
		870.0 (440.0–1870.0)	1890.0 (580.0–3250.0)	1725.0 (380.0–3780.0)
Renal biopsy	Not done	31/44 (70%)	0/26 (0%)	1/18 (5%)
	MCNS	11/44 (25%)	19/26 (73%)	11/18 (61%)
	MCNS with signs of FSGS[a]	2/44 (5%)	7/26 (27%)	6/18 (34%)
Relapses/year		1.04±0.53	1.94±1.53	2.68±2.59
		0.88 (0.24–2.0)	1.39 (0.5–8.0)	1.69 (0.38–8.0)
Dose of prednisone/relapse		312.21±187.48	243.73±72.02	227.0±105.78
		267.0 (110.0–870.0)	232.0 (113.0–416.0)	232.9 (75.0–421.0)

[a]Signs of FSGS in 10–20% of glomeruli

The control group (II) consisted of 18 healthy children (♀-10, ♂-8) aged 2–18 years (9.18±4.7 years), never treated with glucocorticoids. None of the children had any other systemic disorders.

Methods

We measured P-gp expression on peripheral blood T lymphocytes (CD3) of patients with nephrotic syndrome using a flow cytometry assay. P-gp expression was quantified by the percent of P-gp positive cells and absolute number of cells per microlitre. Double (FITC/PE) monoclonal antibodies for immunophenotyping analysis were purchased from Becton Dickinson (BD). All the other reagents were obtained from Coulter. Flow cytometric analysis was performed by Coulter Epics XL in a whole-blood procedure.

The study was approved by the ethics committee of the Medical Academy in Białystok in accordance with the Declaration of Helsinki.

Data analysis was performed with computer program Statistica 6.0. Since no features of normal disintegration were found in the groups examined with the Shapiro–Wilk test, statistical analysis was performed with the non-parametric Mann–Whitney U test or analysis of variance (ANOVA). Spearman correlation coefficients were calculated for statistically significant correlation. A *P* value less than 0.05 was considered statistically significant.

Results

In all subgroups of NS children and also in the control group (male/female −0.8, age 69.18±4.7 years) the children were of similar age and gender ($P>0.05$) (Table 1). Also, the age at evaluation of disease did not differ between the NS subgroups groups ($P>0.05$). The number of NS relapses was significantly higher in FR and SD subgroups.

Total prednisone dose per kilogramme of body weight was the highest in the FR subgroup, a little lower in the SD subgroup and the lowest in the NFR subgroup. The differences were statistically significant. The mean dose of prednisone for the relapse was similar in FR and SD children and higher in NFR children ($P<0.05$). In 32/88 (36.5%) children with NS renal biopsy was not performed, and these were mainly children from the NFR subgroup. In the remaining children minimal change nephrotic syndrome (MCNS) was found in 41/88 (46.5%) and early focal segmental glomerulosclerosis (FSGS) in 15/88 (17%). The ratio MCNS/early FSGS was 19/7 in subgroup FR and 11/6 in SD.

In NFR NS, for 2/44 (5%) children it was the first episode of the illness, in 5/44 (11%) cases it was the first relapse, and in 37/44 (84%) the consecutive relapse.

We evaluated the expression of P-gp on CD3 lymphocytes and total P-gp on other mononuclear cells from children with NS in particular subgroups (Fig. 1 and Table 2). The expression of P-gp in the CD3 lymphocytes (CD3/P-gp), similar to that of total P-gp, was significantly higher than in the control group. The difference was higher in the SD subgroup ($P=0.0001$) (FR $P=0.0002$). The difference in the NFR subgroup was smaller ($P<0.05$).

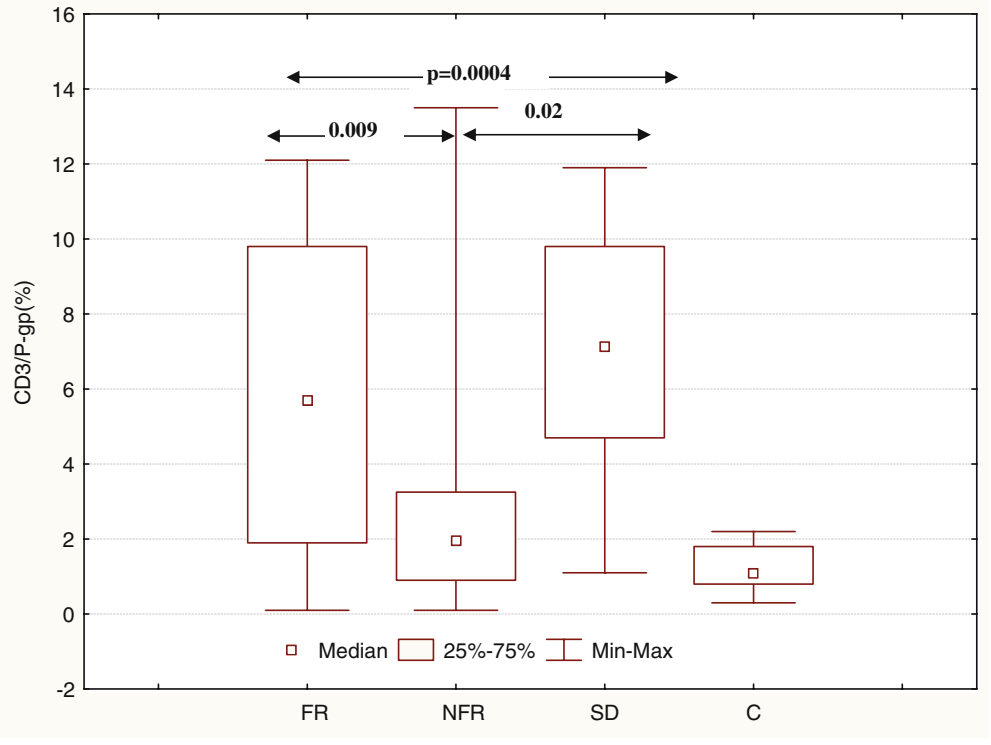

Fig. 1 Expression of P-gp on CD3 lymphocytes of peripheral blood in NS children depending on their clinical response to glucocorticoid (GC) treatment (*FR* frequent relapsers, *NFR* non-frequent relapsers, *SD* steroid - dependent, *C* control)

Table 2 Characteristics of examined parameters in subgroups of children with nephrotic syndrome (I) and in control group (II) (*a.n.* absolute number, *P* comparison with control group)

Group		Number	CD3/P-gp (%)	CD3/P-gp (a.n.)	total P-gp (%)	total P-gp (a.n.)
		Mean ± SD; median (range)				
I	NFR	44	3.02±3.46*	53.01±57.66*	6.19±6.83*	154.6±176.03*
			1.95 (0.1–13.50)	29.95 (1.10–232.8)	4.00 (1.0–34.5)	89.0 (12.0–936.0)
	FR	26	5.56±4.07**	63.96±47.44**	11.47±8.79**	184.65±191.36**
			5.7 (0.1–12.1)	67.04 (1.10–180.7)	11.10 (1.2–34.5)	135 (19.0–908.0)
	SD	18	7.00±3.09**	103.97±77.66**	11.57±9.79**	226.88±264.30**
			7.15 (1.1–11.9)	75.17 (7.98–306.9)	7.05 (0.8–36.5)	153.0 (21.0–706.0)
II		18	1.24±0.58	16.64±8.47	3.40±0.99	66.16±24.44
			1.10 (0.30–2.20)	17.2 (2.49–29.06)	3.25 (1.8–5.3)	70.0 (14.0–100.0)

*$P<0.05$
**$P<0.01$

Mean CD3/P-gp (in percent) was more than twice as high in SD children than in NFR children, and the difference was statistically significant ($P<0.01$). Similarly, the difference between FR and NFR was statistically significant ($P<0.01$). The difference was not significant between subgroups SD and FR ($P>0.05$). Similar results were found in the percentages of total P-gp.

Figure 2 shows the positive correlation between CD3-P-gp and the clinical parameters examined in all NS children. The strongest correlation was observed between CD3/P-gp and number of NS episodes (r=0.305, $P=0.004$). However, a statistically significant positive correlation was also found between CD3/P-gp and total prednisone dose, time to remission and age of onset. In Table 3 we analysed the correlations within each of the subgroups. The correlation between CD3/P-gp and total prednisone dose was the strongest in the SD group, a little weaker in FR and positive, but not statistically significant, in NFR. Correlation with other parameters was similar in the whole group. Only in the FR subgroup did the correlation with the number of relapses become stronger.

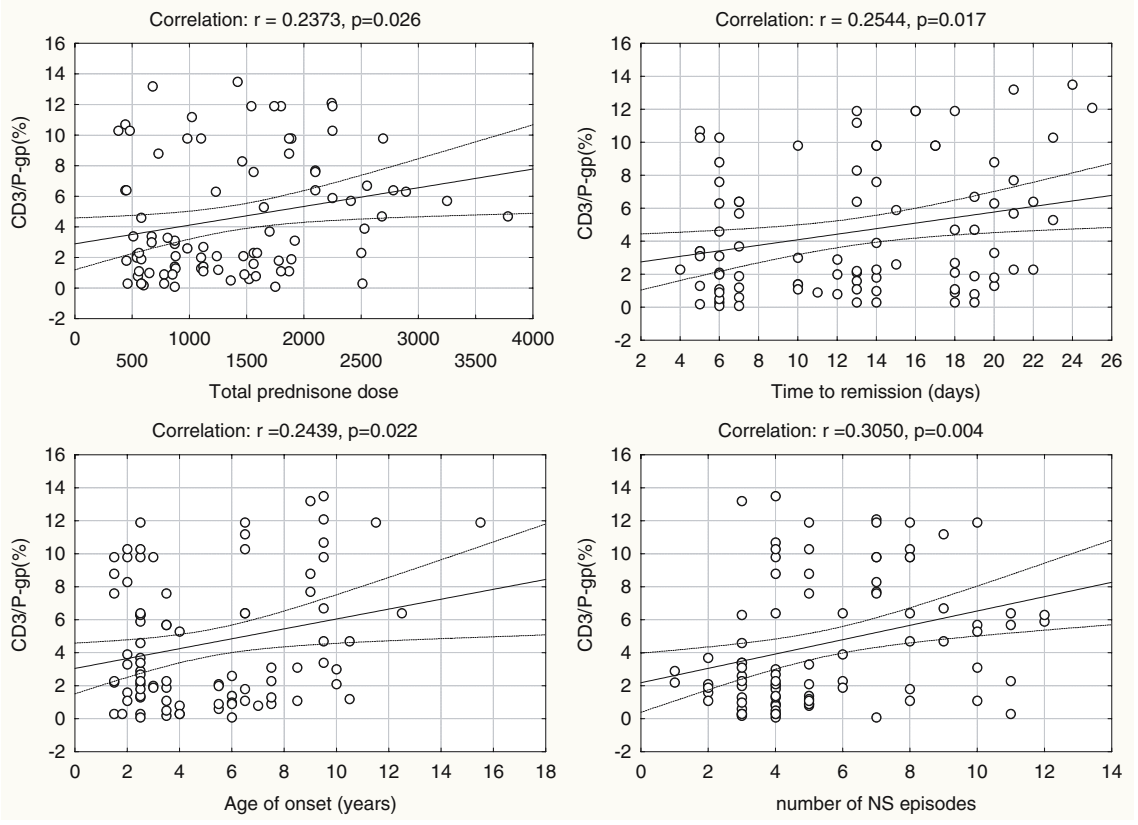

Fig. 2 Correlation between CD3/P-gp expression and (*top left*) total prednisone dose, (*top right*) time to remission, (*bottom left*) age at onset and (*bottom right*) number of NS episodes

Table 3 Correlation between CD3/P-gp expression and total prednisone dose, time to remission, age at onset and number of NS episodes in particular subgroups

CD3/P-gp (%)	Total prednisone dose		Time to remission		Age at onset		Number of NS episodes	
	r	*P*	r	*P*	r	*P*	r	*P*
NFR	0.049	>0.05	0,136	>0.05	0.189	>0.05	0.224	>0.05
FR	0.411	0.037	0.289	0.041	0.266	>0.05	0.458	0.002
SD	0.605	0.008	0.356	0.012	0.341	0.022	0.398	0.003

Discussion

Glucocorticoids are still essential for the control of disease activity in nephrotic syndrome children. Glucocorticoids circulate in the blood in free forms or as complexes with corticosteroid-binding globulin (CBG), called transcortin, or as albumin-binding forms. The biological action of glucocorticoids (GCs) is possible when they shift into target cells [14]. Although children with minimal change nephrotic syndrome initially respond to GC treatment well, some of them show poor response in the course of the disease. They develop frequent relapses or a steroid-dependent course of disease. Several mechanisms may explain glucocorticoid unresponsiveness. Patients with heavy nephrotic syndrome often develop peritonitis or hypoalbuminaemia, which induces glucocorticoid malabsorption from the intestine. Another reason may be rapid glucocorticoid degradation. Among the multiple mechanisms of multidrug resistance, overexpression of P-gp has emerged as the main factor involved in that process. P-gp is a 170 kDa product of the multidrug resistance-1 (MDR-1) gene [15].

The transcription of MDR-1 is directly regulated by human Y-box-binding protein-1 (YB-1), an MDR-1 transcription factor. The activation of YB-1 is induced in response to other multiple drugs, including vinca alkaloids and corticosteroids [16]. Studies in systemic lupus erythematosus (SLE) patients showed that Il-2, a potent lymphocyte stimulator, up-regulates the YB-1 and leads to reduced intracellular steroid concentration [16]. Other authors also found that increased expression of P-gp results in reduction of intracellular concentration of various drugs, including glucocorticoids [17–19].

The expression of P-gp is also modified by cyclosporin A, which is why we excluded children treated with this drug from our examination. The data from literature indicate that cyclosporin A (CyA) is a P-gp substrate and also a competitive inhibitor of it [13, 16]. Therefore, we think cyclosporin A, as a competitor of P-gp, is useful in the treatment of steroid-dependent NS, which was confirmed in clinical trials [20].

In our examination all patients were in clinical remission of nephrotic syndrome. They had no proteinuria, and serum albumin concentration was normal. We assessed the expression of P-gp in CD3 lymphocytes of peripheral blood depending on their clinical response to GC treatment. We found that expression of P-gp was statistically significantly higher in all subgroups of NS children that had been treated with GC in the past than in healthy controls ($P<0.05$). The expression of CD3/P-gp was extremely high in steroid-dependent children. It was almost six-times higher than in the control group. Also, the total P-gp expression was very high in this group of children. Slightly lower CD3/P-gp expression was observed in frequent relapsers. However, the difference in CD3/P-gp expression between these two subgroups was statistically significant. Significantly lower expression was found in non-frequent relapsers, but the difference in comparison to healthy controls was statistically significant ($P<0.05$).

P-glycoprotein expels intracellular drugs, including glucocorticoids [18, 19]. Tsujimura et al. confirmed that P-gp expression on activated lymphocytes resulted in a marked decrease of intracellular glucocorticoid in vitro. Decreased cytoplasm glucocorticoid concentration, a result of increased P-gp-mediated efflux of glucocorticoids from lymphocytes, is one of the mechanism of GC resistance in inflammatory bowel diseases reported by Farrell and Kelleher [21]. Similar suggestions were also made by Montano et al. in steroid-resistant asthma [22]. Szefler, who examined the clinical pharmacology of glucocorticoids in asthma, considers that metabolism of glucocorticoids may be increased by induction of cytochrome P-450 enzymes, which may thus lead to secondary glucocorticoid resistance [23].

Our own analysis of P-gp expression and reports of the authors let us think that it is possible that glucocorticoid therapy may induce P-gp expression. Thus, it is possible that increased P-gp expression in children with SD or FR nephrotic syndrome who received more steroids for longer time may intensify a worse response to steroids or steroid-dependence. It may be also confirmed by clinical observation that children who do not respond to orally administered prednisone often achieve remission after methylprednisolone pulse therapy. It was also demonstrated that CD3/P-gp expression correlated positively with total prednisone dose and number of NS episodes in the whole group of NS children. The correlation was much stronger in children with SD and FR nephrotic syndrome, who had received more steroids during their lifetimes. The correlation in NFR nephrotic children was not statistically significant. It may confirm the suggestion the P-gp function might be induced by the treatment itself. Since one of the main physiological functions of P-gp is detoxification, it is possible that its activity may be a result of high prednisone dosage.

In our recent studies of children with steroid-sensitive nephrotic syndrome, we measured the P-gp expression during prednisone treatment. We found that, prior to

administration of prednisone, P-gp /CD3 expression was a little higher than in the control group, during the prednisone treatment Expression of P-gp increased more than six times and was still increased in remission—8 weeks after glucocorticoid treatment had been finished [24]. Interestingly, the P-gp expression was dependent on the number of NS attacks. In children with many relapses treated with glucocorticoids the P-gp expression was higher than in the control group even after 12-months' remission [24]. A positive correlation between CD3/P-gp expression and time to remission may also be a result of high total steroid dose in children with several relapses. Expression of P-gp showed a positive correlation with the age at onset of illness. The expression was higher in children who were older when the diseases occurred, and they usually responded worse to GC treatment.

Little is known about the role of P-gp in autoimmune diseases. Analysis of P-gp activity in lymphocytes from immune thrombocytopenic purpura (ITP) patients was done by Ruiz-Soto et at. [25]. The authors found that more than 80% of ITP patients showed an increased number of lymphocytes able to expel MDR-1 drugs, suggesting overactivity of P-pg. However, they found no statistical differences among the groups classified according to their clinical response, but it was probably due to small sample size (254). We found two reports in which P-gp function in lymphocytes from SLE patients was evaluated [26, 27]. The prednisone tapering and improvement of clinical status was achieved in parallel with a decrease in MDR-1 lymphocytes [26]. Tsujimura et al. proposed that poor responsiveness to glucocorticoids and immunosuppressants in highly active SLE is caused by P-gp expression on lymphocytes. The authors also suggest that measurement of P-gp on lymphocytes is useful for the prediction of glucocorticoid resistance [27].

On the other hand it may be possible that constitutive high MDR expression may dictate a patient's glucocorticoid requirements. Maillefert et al. [28], in a study of MDR expression in patients with rheumatoid arthritis, found significantly high MDR expression only among patients requiring glucocorticoids. We suggest that it is possible that P-gp expression is a result of MDR-1 gene polymorphisms, which have an influence on P-gp expression, which decreases intracellular GC concentration and causes a worse response to treatment and frequent relapses of NS. Further analysis of MDR-1 polymorphisms in NS children is planned.

In conclusion we still do not have enough data to be sure if increased P-gp expression is a cause of worse steroid response or is only the result of the treatment. In the near future we are going to publish data concerning the MDR1 polymorphism gene in steroid-sensitive, steroid-dependent and steroid resistant children. The data will allow us to determine whether some people are genetically predisposed to better or worse response to steroids.

Conclusion Clinical observation of children with idiopathic nephrotic syndrome shows that steroid-responsiveness during the whole course of diseases is not common. Worse response to glucocorticoids or dependency may be due to overexpression of P-gp. Further examinations are necessary to establish whether increased P-gp expression is a result of MDR1 polymorphism and to determine glucocorticoid response, or to ascertain if such activity is only a result of glucocorticoid therapy. The regulation of P-gp on lymphocytes could provide a novel therapeutic strategy in patients with multidrug resistance.

References

1. Nasi M, Aedelmann CM, Berstein J, Barnett HL (1992) The nephrotic syndrome. In: Edelmann CM, Berstein J, Meadow SR, Spitzer A, Travis LB (eds) Pediatric kidney disease, vol 2. Little, Brown, Boston, pp 1247–1266
2. No authors listed (1981) The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. J Pediatr 98:561–564
3. Ehrich JH, Brodehl J (1993) Long versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. Arbeitsgemeinschaft fur Padiatrische Nephrologie. Eur J Pediatr 152:357–361
4. Leung DJ, Bloom JW (2003) Update on glucocorticoid action and resistance. J Allergy Clin Immunol 111:3–22
5. Hearing SD, Norman M, Probert CS, Haslam N, Dayan CM (1999) Predicting therapeutic outcome in severe ulcerative colitis by measuring in vitro steroid sensitivity of proliferating peripheral blood lymphocytes. Gut 45:382–388
6. Lane SJ, Lee TH (1996) Corticosteroid resistance in other disease states and tissues. Am J Respir Crit Care Med 154: S62–S65
7. Ling V (1997) Multidrug resistance: molecular mechanisms and clinical relevance. Cancer Chemother Pharmacol 40 [Suppl]: S3–S8
8. Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152–162
9. Klimecki WT, Futscher BW, Grogan TM, Dalton WS (1994) P-glycoprotein expression and function in circulating blood cells from normal volunteers. Blood 83:2451–2458
10. Ford JM (1995) Modulators of multidrug resistance. Preclinical studies. Hematol Oncol Clin North Am 9:337–361
11. No authors listed (1982) Early identification of frequent relapsers among children with minimal change nephrotic syndrome. A report of the international study of kidney disease in children. J Pediatr 101:514–518
12. No authors listed (1987) Cyclophosphamide treatment of steroid dependent nephrotic syndrome: comparison of eight week with 12 week course. Report of Arbeitsgemeinschaft für Pädiatrische Nephrologie. Arch Dis Child 62:1102–1106
13. List AF, Kopecky KJ, Willman CL, Head DR, Slovak ML, Douer D, Dakhil SR, Appelbaum FR (2002) Cyclosporine inhibition of P-glycoprotein in chronic myeloid leukemia blast phase. Blood 100:1910–1912
14. Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW (1982) The serum transport of steroid hormones. Recent Prog Horm Res 38:457–510
15. Beck WT, Grogan TM (1997) Methods to detect P-glycoprotein and implications for other drug resistance-associated proteins. Leukemia 11:1107–1109
16. Tsujimura S, Saito K, Nakayamada S, Nakano K, Tsukada J, Kohno K, Tanaka Y (2004) Transcriptional regulation of multidrug resistance-1 gene by interleukin-2 in lymphocytes. Genes Cells 9:1265–1273

17. Tsuruo T (1983) Reversal of acquired resistance to vinca alkaloids and anthracycline antibiotics. Cancer Treat Rep 67: 889–894
18. Bourgeois S, Gruol DJ, Newby RF, Rajah FM (1993) Expression of an mdr gene is associated with a new form of resistance to dexamethasone-induced apoptosis. Mol Endocrinol 7:840–851
19. Meijer OC, de Lange EC, Breimer DD, de Boer AG, Workel JO, de Kloet ER (1998) Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology 139:1789–1793
20. Inoue Y, Iijima K, Nakamura H, Yoshikawa N (1999) Two-year cyclosporin treatment in children with steroid-dependent nephrotic syndrome. Pediatr Nephrol 13:33–38
21. Farrell RJ, Kelleher D (2003) Glucocorticoid resistance in inflammatory bowel disease. J Endocrinol 178:339–346
22. Montano E, Schmitz M, Blaser K, Simon HU (1996) P-glycoprotein expression in circulating blood leukocytes of patients with steroid-resistant asthma. J Investig Allergol Clin Immunol 6:14–21
23. Szefler SJ (1991) Glucocorticoid therapy for asthma: clinical pharmacology. J Allergy Clin Immunol 88:147–165
24. Wasilewska A, Zoch-Zwierz W, Pietruczuk M (2006) Expression of P-glycoprotein in lymphocytes of children with nephrotic syndrome treated with glucocorticoids. Eur J Pediatr (in press)
25. Ruiz-Soto R, Richaud-Patin Y, Lopez-Karpovitch X, Llorente L (2003) Multidrug resistance-1 (MDR-1) in autoimmune disorders III: increased P-glycoprotein activity in lymphocytes from immune thrombocytopenic purpura patients. Exp Hematol 31:483–487
26. Diaz-Borjon A, Richaud-Patin Y, Alvarado de la Barrera C, Jakez-Ocampo J, Ruiz-Arguelles A, Llorente L (2000) Multidrug resistance-1 (MDR-1) in rheumatic autoimmune disorders. Part II: increased P-glycoprotein activity in lymphocytes from systemic lupus erythematosus patients might affect steroid requirements for disease control. Joint Bone Spine 67:40–48
27. Tsujimura S, Saito K, Nakayamada S, Nakano K, Tanaka Y (2005) Clinical relevance of the expression of P-glycoprotein on peripheral blood lymphocytes to steroid resistance in patients with systemic lupus erythematosus. Arthritis Rheum 52:1676–1683
28. Maillefert JF, Maynadie M, Tebib JG, Aho S, Walker P, Chatard C, Dulieu V, Bouvier M, Carli PM, Tavernier C (1996) Expression of the multidrug resistance glycoprotein 170 in the peripheral blood lymphocytes of rheumatoid arthritis patients. The percentage of lymphocytes expressing glycoprotein 170 is increased in patients treated with prednisolone. Br J Rheumatol 35:430–435