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Evaluation of immunologic crossreaction of antiasparaginase antibodies in acute lymphoblastic leukemia (ALL) and lymphoma patients

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To evaluate how well antibodies to one asparaginase preparation predict or correlate with antibodies to another preparation in acute lymphoblastic leukemia (ALL) and lymphoma patients who did and did not have hypersensitivity reactions during chemotherapy. In all, 24 children with newly diagnosed ALL or lymphoma, who received *Escherichia coli* asparaginase 10 000 IU/m^2 IM thrice weekly for nine doses as part of multiagent induction and reinduction chemotherapy, and seven monthly doses during the first 7 months of continuation treatment, were studied. Plasma samples were collected at postinduction and at postreinduction. Six of 24 patients had no overt clinical reactions (nonreacting) and received only the *E. coli* preparation. Of these, 18 patients who had allergic reactions were switched to *Erwinia* asparaginase. A total of 18 patients had an anaphylactoid reaction to *Erwinia* asparaginase and were switched to receive polyethylene glycol (PEG) asparaginase. Antibody levels were measured by enzyme-linked immunoadsorbent assay against all the three asparaginase preparations. At postinduction, antibodies against *E. coli* were higher in reacting patients (0.063$\pm$0.066) than in nonreacting patients (0.019$\pm$0.013) (P=0.03). At postreinduction, anti-*Erwinia* antibodies were significantly higher in reacting patients (0.431$\pm$0.727) than in nonreacting patients (0.018$\pm$0.009) (P=0.007). Anti-*E. coli* antibodies correlated with anti-PEG antibodies at postinduction (r=0.714, P<0.001) and at postreinduction (r=0.914, P<0.001), but did not correlate with anti-*Erwinia* antibodies at postinduction (r=0.119, P=0.580) and at postreinduction (r=0.078, P=0.716). The results indicate a crossreactivity between patient antibodies raised against natural *E. coli* and PEG asparaginase but not *Erwinia* asparaginase.
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Introduction

Asparagine is considered a nonessential amino acid, but certain leukemic cells are unable to synthesize sufficient quantities of asparagine due to a diminished asparagine synthetase activity.[1] Thus, the leukemic cells depend on extracellular sources of asparagine to complete protein synthesis. Asparaginase catalyzes the hydrolysis of asparagine to aspartic acid and ammonia in the extracellular fluid.[2] As a result of asparagine depletion, asparaginase exploits a metabolic difference between normal cells and malignant cells to diminish the synthesis of protein and nucleic acids in leukemic cells.[2–6] Thus, asparaginase is a critical agent in the treatment of childhood acute lymphoblastic leukemia (ALL) and lymphoma.[2,3,7]

Of the three asparaginase products available in the United States, *Escherichia coli* asparaginase is isolated from *E. coli*, *Erwinia* asparaginase is isolated from *Erwinia chrysanthemi*, and pegaspargase asparaginase is modified from *E. coli* asparaginase with covalent linkage to polyethylene glycol (PEG). Since asparaginase is a foreign protein, the development of antiasparaginase antibodies frequently occurs. Antiasparaginase antibodies may cause a decreased asparaginase activity through several mechanisms. Antiasparaginase antibodies have been associated with increased drug clearance thus shortening its half-life,[8,9] decreasing circulating enzyme activity,[10,11] or lowering enzyme activity by deposition of antigen–antibody complex in the reticuloendothelial system.[12] Asparaginase-specific IgG antibodies appear to correlate with clinical allergic reactions.[13,14]

The severity of hypersensitivity reactions to the three asparaginase preparations ranges from mild allergic reactions to anaphylactic shock.[7,14–20] The reported frequency of antiasparaginase antibodies was as high as 78% in adults and 70% in children following intravenous or intramuscular administration of *E. coli* asparaginase.[21] In all, 23–30% of children developed allergic reaction while receiving *Erwinia* asparaginase.[14,17] Allergic reaction was observed in 30–41% of patients who received PEG asparaginase.[18,22] Recently, the importance of 'silent hypersensitivity' has been recognized in patients who developed antiasparaginase antibodies without clinical evidence of a hypersensitivity reaction.[6,9,12,21] Investigators found that those patients had a significant reduction in plasma asparaginase activity[9] and suboptimal asparagine depletion.[23]

Allergic reactions to one preparation can be circumvented in most patients by the substitution to another preparation. In the United States, *E. coli* asparaginase is the most commonly used preparation. Hypersensitivity reactions usually require discontinuation of *E. coli* asparaginase and substitution with the *Erwinia* preparation.[6,7,14] The properties of PEG asparaginase were intended to reduce the immunogenicity and the risk of hypersensitivity reactions and to prolong the half-life of the drug.[24] Thus, PEG asparaginase has been used in patients who have prior allergy to *E. coli* or *Erwinia* asparaginase.[6,25]

It is not clear whether antibodies to *E. coli* asparaginase also exhibit crossreactivity to *Erwinia* asparaginase or PEG asparaginase. To answer this question, we determined antiasparaginase antibody levels to *E. coli*, Erwinia, and PEG asparaginase preparations in 22 newly diagnosed childhood ALL and two newly diagnosed lymphoma patients who did and did not have hypersensitivity reactions during chemotherapy. We report the crossreactivity of antiasparaginase antibodies against three enzyme preparations at identical time points relative to therapy in ALL patients.

Patients and methods

Patient selection

This study was performed in 22 children with newly diagnosed ALL and two patients with newly diagnosed non-Hodgkin's

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lymphoma (NHL) (total of 24 patients) enrolled on the St Jude Children's Research Hospital protocols for ALL (Total XIIIH) and NHL (NHL XIII), respectively. Patients were less than 18 years of age (median: 4.8 years) and previously untreated with asparaginase. The criteria for eligibility, diagnosis, risk-group classification, and ALL treatment have been reported elsewhere.[26] NHL therapy was identical, except that all patients (instead of half in the ALL patients) received high-dose methotrexate (1 g/m^2 over 24 h) prior to conventional remission induction therapy. The study group included patients who eventually had clinical reactions to asparaginase and who had samples available at both induction and reinduction treatments. Clinical characteristics of the allergic reactions included erythema, pruritus, swelling, and pain at the side of injection, or urticaria, angioedema, wheezing, fever, vomiting, cyanosis, and respiratory distress. Antibody data using only *E. coli* as the antigen for the 22 ALL patients were previously presented.[14]

Description of chemotherapy regimens

E. coli asparaginase (Elspar®, Merck & Co., West Point, PA, USA) was administered at a dose of 10 000 IU/m^2 intramuscularly three times weekly for a total of nine doses during both induction and reinduction phases and seven monthly doses during the first 7 months of continuation therapy. The multiagent treatment regimen for total XIIIH has been reported elsewhere.[14] In cases of mild asparaginase allergy, patients were to be premedicated with diphenhydramine with or without glucocorticoids. In subsequent or more severe cases, patients were switched to *Erwinia* asparaginase with the same dosage and scheduling intramuscularly as *E. coli* asparaginase. Patients with an allergic reaction to *Erwinia* were switched to PEG asparaginase at a dose of 2500 IU/m^2/week intramuscularly. Informed consent was obtained from patients' parents or guardians according to Institutional Review Board guidelines.

Laboratory materials

The *E. coli* enzyme (Elspar®, lot no. 0860D) was obtained from Merck & Co., West Point, PA, USA. The *Erwinia*-derived asparaginase (*Erwinia*, lot no. 1009H6E) was manufactured by *MRA*/CAMR, Salisbury, England. The PEG asparaginase product (Oncospar®, lot no. A02603), which was the *E. coli* enzyme modified by covalent attachment of PEG groups, was manufactured by Enzon, Inc., Piscataway, NJ, USA. A Thermomax microplate reader (Molecular Devices Corp., Menlo Park, CA, USA) was used to measure the absorbance for all enzyme-linked immunoadsorbent assay (ELISA) experiments using the manufacturer's software. Microplates (Nunc-Immuno plate, cat. no. 62409–50) were purchased from Thomas Scientific Products. All other materials were obtained from Sigma Chemical Co (St Louis, MO, USA), unless otherwise indicated.

Determination of antiasparaginase antibodies

Antiasparaginase antibody levels were measured by ELISA as previously reported.[27] The method validation for the specificity and the sensitivity was detailed in the paper. Briefly, *E. coli* (5 g/ml), *Erwinia* (5 g/ml), and PEG (10 g/ml) asparaginase were coated in the wells of the plate. The positive antiasparaginase antibody control, normal human plasma (NHP) negative control, and patient plasma samples at different dilutions were then added and incubated for 1 h, after which a polyclonal goat anti-human IgG horseradish peroxidase conjugate was added and incubated for 1 h. After washing, *o*-phenylenediamine dihydrochloride was added and incubated for 30 min. Antiasparaginase antibody levels were measured at 490 nm for the enzymatic product (subtracting the absorbance at 650 nm for nonspecific absorbance) using a microplate reader. When the optical density (OD) readings of the positive and the negative controls were in the acceptable range, the antibody levels of patient samples were recorded as the mean of the OD values from duplicate wells. Positive reactivity in plasma was designated as OD values greater than the mean plus two standard deviations of the OD values determined from 32 NHP controls (ie OD >0.042 for anti-*E. coli* antibodies OD >0.093 for anti-*Erwinia* antibodies; and OD$>$ 0.033 for anti-PEG antibodies).

Evaluation of antiasparaginase antibody crossactivity

Peripheral blood samples were collected in heparinized tubes at the end of induction (ie 10 days after the ninth dose of *E. coli* asparaginase) and at the end of reinduction (ie after 25 planned doses of asparaginase). Samples were centrifuged at 3000 r.p.m. for 5 min, and the plasma was stored at -70°C until analysis. Six of 24 patients who only received *E. coli* preparation were designated as the nonreacting group. Five of them did not have any clinical reaction and one had a mild reaction but completed all doses of asparaginase treatment with the *E. coli* asparaginase. Of the 24 patients who switched to *Erwinia* due to allergic reactions, 18 were designated as the reacting group. One of 18 patients with an anaphylacitiod reaction to *Erwinia* was switched to PEG asparaginase. The plasma antibody levels were tested against *E. coli*, *Erwinia*, and PEG asparaginase preparations. The crossreactivity of anti-*E. coli* asparaginase antibodies in plasma was designated as OD values greater than 0.093 for anti-*Erwinia* antibodies and greater than 0.033 for anti-PEG antibodies.

Statistical analysis

Anti-*E. coli*, *Erwinia*, and PEG asparaginase antibody levels at postinduction were compared to those at postreinduction using a Wilcoxon matched-pair test. Antibody levels against *E. coli*, *Erwinia*, and PEG asparaginase between groups (those who developed allergy *vs* those who did not) after both induction and reinduction were compared with a Mann–Whitney *U*-test. The correlation of antibodies to one asparaginase preparation with antibodies to another preparation in patients who did and did not have hypersensitivity reactions during chemotherapy of ALL was determined by Spearman's *r* correlation (Statistica for Windows, Statsoft, Tulsa, OK, USA).

Results

Patient characteristics and toxicity

Table 1 summarizes the characteristics of 24 patients according to the occurrence of clinical allergic reactions. The schedule of asparaginase preparations and the occurrence of clinical allergic reactions for 24 patients is summarized in Table 2. All six patients in the nonreacting group completed 25 planned doses of *E. coli* asparaginase. Of 18 reacting patients, 13 patients

Table 1 Patient characteristics according to occurrence of allergic reactions

Characteristic	*Reacting patients*	*Nonreacting patients*
No. of patients	18	6
Gender		
Male	15	4
Female	3	2
Median age (years)	4.8	4.5
Range	0.9–17.2	2.8–15.1
Race		
White	14	4
Black	2	2
Hispanic	2	0
Immunophenotype		
B lineage	16	5
T lineage	0	1
Lymphoma	2	0

completed 25 doses, whereas two patients received 21 doses, one received 23 doses and one 24 doses due to a protocol modification (by deleting asparaginase during continuation therapy). One patient only received a total of 15 doses (10 doses of *E. coli* and five doses of *Erwinia*) due to allergic reactions.

In the nonreacting group, five patients had no clinical allergic reaction and one patient had an allergic reaction at the 20th dose during the reinduction, but, because this patient was not changed to *Erwinia* he was counted in the nonreacting group.

Following an allergic reaction to *E. coli* asparaginase, patients either continued on the same preparation with premedication or were switched to the *Erwinia* asparaginase product. In the reacting group, all patients were eventually switched to receive *Erwinia* asparaginase. Initial allergic reactions developed after four to 17 doses (median, 11.7) of *E. coli* asparaginase (Table 2). The first allergic reaction occurred with the fourth *E. coli* asparaginase dose ($n=1$), the 10th dose ($n=4$), the 11th dose ($n=4$), the 12th dose ($n=5$), the 13th dose ($n=2$), the 14th dose ($n=1$), and the 17th dose ($n=1$). After the initial reaction to *E. coli* asparaginase, 10 patients continued to receive the same preparation (with diphenhydramine or corticosteroid premedication) and eight patients were switched to *Erwinia* asparaginase. However, all patients who continued on *E. coli* asparaginase eventually reacted again, and were subsequently switched to the *Erwinia* asparaginase. One patient exhibited a clinical anaphylacitoid reaction to *Erwinia* asparaginase and was switched to receive PEG asparaginase for a single week at the end of reinduction therapy.

Table 2 The asparaginase schedules and the occurrence of clinical allergic reactions

	Induction (3 weeks)									Continuation (7 months)							Reinduction (3 weeks)									
	1			2			3			4	5	6	7	8	9	10	11			12			13			Total
Asnase dose #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Doses
Reacting																										
1	Ec[1]											R[4]		▶	Er[2]										▶	25
2	Ec									R						▶	Er								▶	25
3	Ec										R							▶	Er						▶	25
4	Ec											R				▶	Er							▶		24
5	Ec										▶R	Er		R											▶	25
6	Ec											R			▶	Er			R						▶	25
7	Ec			R						▶	Er				▶											15
8	Ec													▶R	Er-R										▶	25
9	Ec											R				▶	Er			R					▶	25
10	Ec									▶R	Er														▶	25
11	Ec										▶R	Er													▶	25
12	Ec									R		▶	Er												▶	25
13	Ec											▶R	Er												▶	25
14	Ec																▶R	Er					▶			23
15	Ec												R	▶	Er										▶	25
16	Ec										R						▶	Er							▶	25
17	Ec												▶R	Er							▶					21
18	Ec									▶R	Er						R			▶	Peg[3] ▶					21
Non-reacting																										
1	Ec																								▶	25
2	Ec																								▶	25
3	Ec																								▶	25
4	Ec																								▶	25
5	Ec																								▶	25
6	Ec																			R					▶	25

1. Ec = *E.coli* asparaginase; 2. Er = *Erwinia* asparaginase; 3. Peg = PEG asparaginase; 4. R = the day that patient had a clinical reaction.

Antiasparaginase antibody concentrations

Samples collected at the end of induction and at the end of reinduction were assayed against *E. coli*, *Erwinia*, and PEG antigens. The antibody levels directed against all these antigens at postreinduction in 24 patients were significantly higher compared to those at postinduction (anti-*E. coli* antibodies: 0.729 ± 0.560 *vs* 0.052 ± 0.060, $P<0.001$; anti-*Erwinia* antibodies: 0.332 ± 0.640 *vs* 0.031 ± 0.017, $P=0.050$; anti-PEG antibodies: 0.165 ± 0.243 *vs* 0.014 ± 0.010, $P<0.001$), respectively.

We compared antibodies to *E. coli*, *Erwinia*, and PEG antigens between the reacting and the nonreacting patients in Table 3. At the end of induction, all patients had only received *E. coli* asparaginase. Antibodies against *E. coli* were higher in the reacting group (0.063 ± 0.066) than those in the nonreacting group (0.019 ± 0.013) ($P=0.030$), but anti-*Erwinia* and PEG asparaginase antibody levels showed no significant difference between the two groups. By the end of reinduction, 18 patients who had a reaction were switched to *Erwinia* asparaginase. Anti-*Erwinia* antibodies were higher in the reacting patients (0.431 ± 0.727, $n=18$) than those in the nonreacting patients (0.018 ± 0.009, $n=6$) ($P=0.007$), whereas anti-*E. coli* and PEG asparaginase antibodies were not significantly different in the two groups at the end of reinduction.

Crossreactivity of antiasparaginase antibody levels

All 24 patients only received *E. coli* asparaginase when the first sample was collected at the end of induction. Figure 1 illustrates the crossreactivity of patient plasma against *E. coli, Erwinia*, and PEG. At the end of induction, when all of the patients had only received *E. coli*, 29% (7/24) reacted with *E. coli*, 17% (4/24) crossreacted with PEG, and none (0/24) of them crossreacted with the *Erwinia* antigen. When the second sample was collected at the end of reinduction, 18 patients had been switched to *Erwinia* asparaginase due to clinical allergic reactions, and six patients remained on *E. coli* asparaginase. At the end of reinduction, 94% (17/18) of the reacting patient's plasma reacted with *E. coli*, 33% (6/18) reacted with *Erwinia*, and 72% (13/18) reacted with PEG asparaginase. The plasma of all six (100%) nonreacting patients, who only received *E. coli* during therapy, reacted with *E. coli* antigen, and crossreacted with PEG asparaginase in 67% (4/6), but none (0/6) of the plasma crossreacted with *Erwinia* asparaginase.

Figure 2 shows the correlation of antibodies to *E. coli* asparaginase *vs* Erwinia or PEG preparation in 24 patients. Anti-*E. coli* antibodies correlated with anti-PEG antibodies at the end of induction ($r=0.714$, $P<0.001$) and at the end of reinduction, $r=0.914$, $P<0.001$). However, anti-*E. coli* antibodies did not correlate with anti-*Erwinia* antibodies at either time ($r=0.119$, $P=0.580$ at the end of induction; and $r=0.078$, $P=0.716$ at the end of reinduction).

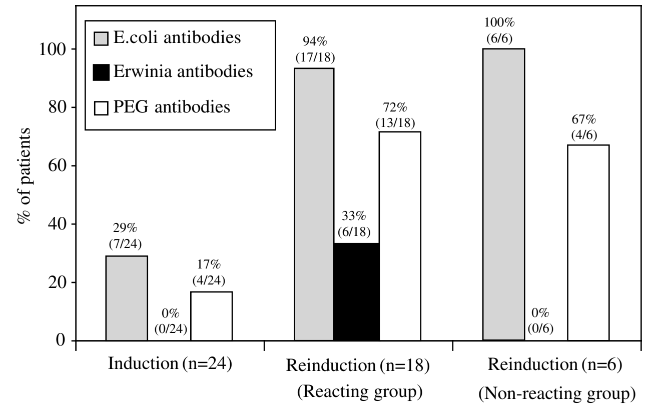

Figure 1 Proportion of antiasparaginase antibody crossreaction against *E. coli*, *Erwinia,* and PEG asparaginase antigens at the end of induction and reinduction. All of 24 patients only received *E. coli* asparaginase at the end of induction. At the end of reinduction, 18 patients received *E. coli* and *Erwinia*; one patient received *E. coli*, *Erwinia*, and PEG in the reacting group. Six patients only received *E. coli* asparaginase in the nonreacting group at the end of reinduction.

Table 3 Comparison of antibody levels according to occurrence of allergic reaction

	Reacting patients[a]	*Non-reacting patients*[b]	*P*-values[c]
	(*n*=18) OD (mean±s.d.)	(*n*=6) OD (mean±s.d.)	
Induction (all patients received only *E. coli*)			
E. coli	0.063±0.066	0.019±0.013	0.030
Erwinia	0.034±0.018	0.023±0.013	NS
PEG	0.015±0.012	0.010±0.013	NS
Reinduction			
E. coli	0.723±0.587	0.747±0.602	NS
Erwinia	0.431±0.727	0.018±0.009	0.007
PEG	0.134±0.155	0.217±0.312	NS

[a]In all, 17 patients received *E. coli* and *Erwinia*, one patient received *E. coli*, *Erwinia*, and PEG.
[b]Six patients received only *E. coli*.
[c]Antibody levels in reacting patients were compared to that in nonreacting patients using
Mann–Whitney *U*-test.
NS, not significant.

Discussion

L-asparaginase is an effective drug in the treatment of ALL. A major toxicity of L-asparaginase is hypersensitivity, which may attenuate the pharmacological effect of the drug. In this study, we found that patients (reacting group, $n=18$) who eventually developed allergic reactions, had higher levels of anti-*E. coli* antibody than those (nonreacting group, $n=6$) who did not have allergic reaction at the end of induction. However, there was no significant difference in antibody levels against *Erwinia* and PEG between the reacting and the nonreacting groups at the end of induction. After having an allergic reaction, 18 patients were switched to *Erwinia* asparaginase for their remaining therapy. At the end of reinduction, patients who switched to *Erwinia* asparaginase had significantly higher anti-*Erwinia* antibody levels than those who did not. At the end of reinduction period, patients with anti-*E. coli* antibodies also had anti-PEG antibodies even though they did not receive PEG asparaginase.

In order to test our hypothesis of the crossreaction among the three asparaginase preparations, we tested 32 NHP samples. To determine baseline OD readings, we then defined the positive antibodies as OD values greater than the mean plus two standard deviations above the OD values in 32 NHP (data not shown). Then, we assayed baseline samples from 31 ALL patients before they received asparaginase. Only one OD value of 31 ALL predose samples was higher than the OD value of 0.43 for anti-*E. coli* antibodies. None of 31 ALL predose samples

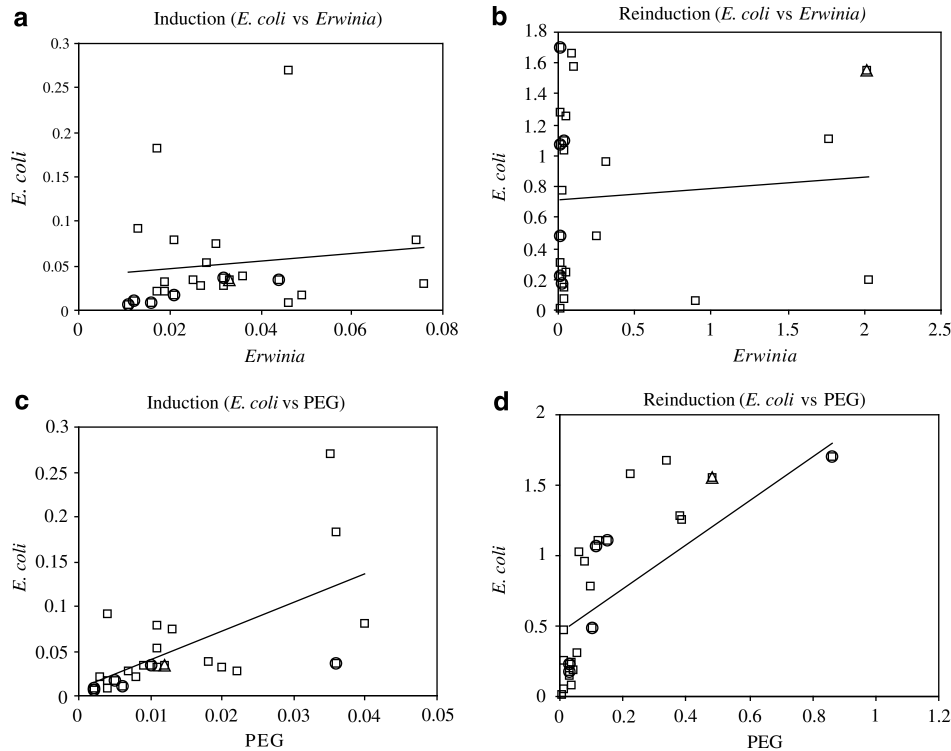

Figure 2 No correlation between *Erwinia vs E. coli* at induction (**a**) ($r=0.119$, $P=0.580$) and at reinduction in (**b**) ($r=0.078$, $P=0.716$). Correlation between PEG *vs E. coli* asparaginase at induction (**c**) ($r=0.714$, $P<0.001$) and at reinduction (**d**) ($r=0.914$, $P<0.001$).

were > 0.93 for anti-*Erwinia* antibodies or >0.33 for anti-PEG antibodies (data not shown). Thus, those OD values were established as positive results.

Antibodies are capable of expressing remarkable specificity and are able to distinguish between small differences in the primary amino-acid sequence of protein antigens, as well as differences in charge and optical configuration.[28] Since they are derived from different bacterial sources, the antigenic sites are different between *E. coli* and *Erwinia chrysanthemi* species.[6] PEG asparaginase is a modified *E. coli* asparaginase with two PEG chains.[8] Therefore, *E. coli* and PEG asparaginase may be expected to have similar antigen epitopes. In this study, we found that *E. coli* asparaginase-directed antibodies, exhibited significant crossreactivity when tested against PEG asparaginase and that this crossreactivity increased in the postreinduction period when compared to the postinduction period for both reacting and nonreacting groups. However, anti-*Erwinia* antibody levels were not correlated with anti-*E. coli* antibody levels.

Although pegylation of asparaginase may make the molecule less antigenic, anaphylaxis has not been eliminated by PEG-modified asparaginase as originally thought.[18,19,22,29] When 126 patients received subsequent treatment with PEG asparaginase intramuscularly, hypersensitivity reactions occurred in 30% of patients who had not previously experienced a hypersensitivity reaction to *E. coli* asparaginase and in 11% of patients who had no prior hypersensitivity to *E. coli* asparaginase.[22] In addition, Asselin *et al*[8] found that serum PEG enzyme activity decreased significantly faster in patients who had allergic reactions to *E. coli* asparaginase than in patients who had not previously received any asparaginase preparation. The spectrum of toxicity of PEG asparaginase is similar to that of *E. coli* asparaginase.[26,30,31] In this study, we observed that the anti-*E. coli* antibodies crossreacted to PEG in the plasma samples at the end of induction and at the end of reinduction. Although not measured in this study, the crossreactivity between *E. coli* and PEG asparaginase may be expected to be associated with allergic reactions during subsequent treatment with PEG asparaginase. We have shown the crossactivity of anti-*E. coli* antibodies with PEG only in one direction. *E. coli* asparaginase is the first choice of the three preparations in the most of protocols in United States. Therefore, we cannot make an assumption that the antibodies developed against PEG asparaginase will crossreact with native *E. coli* based on the data obtained in this study.

Although the number of patients entered in our study is relatively small, our results clearly indicate the crossreactivity between *E. coli* and PEG asparaginase. Despite the fact that patients who had reactions were switched from *E. coli* to *Erwinia*, there was greater crossreactivity of plasma against *E. coli* and PEG than there was between *E. coli* and *Erwinia*. If the development of antibodies corresponds with inactivation of asparaginase, these findings support the practice of using *Erwinia* asparaginase as the substituted agent for patients who have allergic reactions to *E. coli*. The serum half-lives of the three asparaginase preparations are 5.7 days for PEG, 1.3 days native *E.coli*, and 0.7days *Erwinia*.[9] The differences in half-lives were reflected in the duration of serum asparagine depletion. Duval *et al*[32] compared *E. coli* to *Erwinia* asparaginase at equal doses of 10 000 IU twice weekly intravenously in childhood lymphoid malignancies. The results indicated that the clinical efficacy of *E. coli* asparaginase is higher than that of *Erwinia* asparaginase and the toxicity of inducing coagulation abnormalities with *E. coli* asparaginase is higher than that with *Erwinia* asparaginase. The authors recommended *E.coli* asparaginase for first-line therapy and *Erwinia* asparaginase as the substitution agent in allergic patients.[32] It is necessary to indicate the specific

commercial product used in multicenter trails as the doses and schedules may be varied with different preparations.[8,9,33] Whenever possible a program should be established to monitor the asparagine levels in plasma and CSF. In case where an alternative asparaginase preparation is used, patients should be carefully monitored to achieve complete depletion of asparagine in plasma and CSF.[34]

In conclusion, the results indicate a crossreactivity between antibodies directed against natural *E. coli* and PEG asparaginase. It is not known if such crossreactivity *in vitro* will translate into crosshypersensitivity or crossinactivation *in vivo*.

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