



Anti-asparaginase antibodies following *E. coli* asparaginase therapy in pediatric acute lymphoblastic leukemia

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Asparaginase is an effective antileukemic agent and is included in most front-line protocols for pediatric acute lymphoblastic leukemia (ALL) worldwide; however, allergic reactions to asparaginase may be dose-limiting. We evaluated plasma anti-asparaginase antibody concentrations in a cohort of children with newly diagnosed ALL, who did and who did not exhibit clinical hypersensitivity, after *Escherichia coli* (*E. coli*) asparaginase therapy. Thirty-five children who received asparaginase 10 000 IU/m² i.m. three times weekly for nine doses as part of both multiagent induction and reinduction chemotherapy, and seven monthly doses during the first 7 months of continuation treatment, were studied. Twenty-two patients experienced initial allergic reactions to asparaginase during continuation ($n=20$) or reinduction ($n=2$) phases and 13 children did not exhibit any reaction. An enzyme-linked immunosorbent assay (ELISA) was used to measure anti-asparaginase antibodies in plasma samples, diluted 1:3200, using *E. coli* asparaginase as the antigen. The median anti-asparaginase antibody concentration (OD at 1:3200 dilution) increased from 0.039 at induction to 0.506 at reinduction in patients who exhibited clinical hypersensitivity ($P=0.0002$). By comparison, median antibody level increased from 0.011 to 0.032 OD at identical time points in patients who did not react to asparaginase ($P=0.02$). Both post-induction and post-reinduction anti-asparaginase antibody levels were higher in reacting than in nonreacting patients ($P=0.004$ and $P=0.01$, respectively). Antibody levels were inversely related to the time elapsed between the reaction and sampling ($P=0.011$). Although anti-asparaginase antibody levels increased from the post-induction plasma sample to the post-reinduction sample in 28 of 35 patients regardless of whether they exhibited clinical hypersensitivity, patients with hypersensitivity reactions had higher antibody levels than did identically treated control patients at comparable time points in therapy. Therefore, antibody analysis may be of clinical value in predicting future hypersensitivity.

Keywords: asparaginase; antibodies; allergic reaction; acute lymphoblastic leukemia

Introduction

Asparaginase has been used to treat leukemia for over 25 years. It exploits a metabolic difference between normal cells and malignant cells. Normal cells can synthesize asparagine as needed; however, asparagine synthetase activity in some malignant lymphoblasts is low, and thus endogenous production of this amino acid is diminished.^{1,2} Asparaginase hydrolyzes asparagine to aspartic acid and ammonia extracellularly, depriving neoplastic cells of their normal source of asparagine. Asparagine-dependent protein synthesis is halted with subsequent inhibition of nucleic acid synthesis, which decreases leukemic cell proliferation.

Because asparaginase is not expressed in humans, but is isolated from bacterial sources, one of the primary limitations

to its use is the development of anti-asparaginase antibodies. Available pharmaceutical preparations of asparaginase vary in their pharmacokinetic and immunogenic properties.^{3–5} Asparaginase is isolated from two primary sources: *Escherichia coli* and *Erwinia chrysanthemi*. The former is the source of the most commonly used preparation in the United States and is also widely used throughout the world. PEG asparaginase is a conjugate of the native *E. coli* asparaginase covalently linked to polyethylene glycol, which may decrease the probability of developing anti-asparaginase antibodies^{6,7} and prolongs the elimination half-life of the drug.³

Hypersensitivity reactions to *E. coli* preparations given intravenously or intramuscularly are dose-limiting in 4–45% and 0–25% of patients, respectively. These reactions usually necessitate discontinuation of *E. coli* asparaginase and subsequent substitution with *Erwinia* asparaginase. This latter preparation is equally allergenic;^{4,8} however, it exhibits limited, but clinically relevant, immunologic cross-reactivity.⁹

It has been suggested that development of antibodies may hamper the antileukemic effect of asparaginase by shortening its half-life, preventing or delaying absorption after intramuscular injection, or interfering with enzyme activity.^{3,10,11} The reported frequency of anti-asparaginase antibodies is quite variable, and so high in some subgroups of patients (ie over 70% in adults receiving *E. coli* asparaginase)^{10,12} that it is not clear whether antibody levels are more frequently detected or higher in patients who exhibit overt clinical allergy than in identically treated patients without clinical evidence of hypersensitivity. Therefore, the objective of this study was to retrospectively compare anti-asparaginase antibody concentrations at identical time points relative to therapy in ALL patients who did and who did not develop hypersensitivity reactions to asparaginase. These are the first data on anti-asparaginase antibodies at fixed time points relative to asparaginase dosing in patients with newly diagnosed ALL.

Patients and methods

Patient eligibility and treatment schedules

Thirty-five children with newly diagnosed ALL enrolled on the St Jude Children's Research Hospital front-line protocol (Total XIIIH) and with frozen plasma available at both the end of induction and reinduction phases were evaluated in this study. The criteria for eligibility, diagnosis, risk-group classification, and treatment have been reported elsewhere.¹³ Twenty-two patients who exhibited clinically overt hypersensitivity reactions to asparaginase were compared to 13 children without reactions. All allergic reactions were graded using the standard NCI common toxicity criteria. As per protocol, *E. coli* asparaginase (Elspar, Merck, West Point, PA, USA) was administered at a dose of 10 000 IU/m² intramuscularly three times weekly for a total of nine doses during both induc-

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tion and reinduction phases, and seven monthly doses during the first 7 months of continuation therapy. The multiagent treatment regimen for Total XIIIH is shown in Table 1. 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 to be switched to *Erwinia* asparaginase. Those with anaphylactic reactions to *Erwinia* were to be switched to PEG asparaginase. Informed consent was obtained from patients' parents according to Institutional Review Board guidelines.

Sample collection

Blood samples from a peripheral vein were collected in heparinized tubes post-induction therapy on day 29 of treatment (ie 10 days after nine doses of asparaginase, but before any allergic reaction) and on day 22 of reinduction (ie after 25 planned doses of asparaginase, and, in patients with hypersensitivity, after the allergic reaction). Samples were centrifuged at 3000 r.p.m. for 5 min, and the plasma was stored at -70°C until analysis.

Determination of anti-asparaginase antibodies

Anti-asparaginase antibody concentrations were measured by ELISA. *E. coli* asparaginase (Elspar, 10 000 IU, Lot No. 0496D, Merck) was diluted to 5 $\mu\text{g}/\text{ml}$ in 0.05 M carbonate-bicarbonate buffer, pH 9.4. One hundred microliters were added to 96-well polystyrene ELISA plates (F96 Poly-Sorp Nunc-Immuno Plate; VWR Scientific Product, Suwanee, GA, USA) and incubated at $2-8^{\circ}\text{C}$ overnight. The excess antigen was drained, and 300 μl of 0.1% bovine serum albumin (RIA Grade) (Sigma, St Louis, MO, USA) in phosphate-buffered saline was added to each well and incubated at room temperature for at least 90 min. The wells were drained and washed with Saline-Tween (0.145 M NaCl; 0.05% Tween 20) three times. Patient plasma was serially diluted at 1:400, 1:800, 1:1600, and 1:3200 in Saline-Tween, and 100 μl was added in duplicate wells and incubated at room

temperature for 1 h. Each 96-well plate included negative controls (normal human plasma and diluent alone) and positive controls (pooled human plasma collected within 0–7 days of a grade 3–4 allergic reaction to *E. coli* asparaginase) at similar dilutions. Wells were then drained and washed with Saline-Tween three times, and developed with 100 μl of horseradish peroxidase-conjugated goat anti-human IgG (Sigma) diluted 1:10 000 in Saline-Tween. After a 1 h incubation at room temperature, the plates were washed four times with Saline-Tween. One hundred microliters of substrate solution (20 mg of *o*-phenylenediamine $\cdot$ 2HCl (Sigma), 167 μl of 3% hydrogen peroxide, and 50 ml of 0.1 M citrate-phosphate buffer) was added to each well and incubated for 30 min at room temperature in the dark, and afterwards 100 μl of 1 M phosphoric acid was added to stop the reaction. Individual wells were read on an ELISA reader set to read optical density (OD) at a wavelength of 490 nm minus the OD at 650 nm (Molecular Devices, Sunnyvale, CA, USA). Data are reported as OD readings at the 1:3200 dilution. In addition, anti-asparaginase antibody concentration in the pooled human plasma used for the positive control was quantitated; daily and day-to-day coefficients of variation (CV) were calculated. Intraday CV values from samples assayed in triplicate on 3 separate days were $<5\%$; the mean antibody level used to calculate daily CVs was the same as that used to determine interday variability (ie 23 measurements over 5 months). Day-to-day variability in antibody level evaluated in duplicate or triplicate over 5 months was also $<5\%$.

Statistical analysis

Post-induction anti-asparaginase antibody levels were compared to post-reinduction levels using a Wilcoxon matched pairs test. Antibody levels 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 relationship between anti-asparaginase antibody level and time between the last allergic reaction and time of sampling was determined by Spearman R correlation (Statistica for Windows, Statsoft, Tulsa, OK, USA).

Table 1 Treatment regimen for study Total XIIIH

Remission induction/reinduction	
Weeks 0–4	Methotrexate, either 1 g/m^2 i.v. $\times$ 24 h or 30 mg/m^2 p.o. q 6 h $\times$ 6, followed in 96 h by prednisone 40 mg/m^2 p.o. daily for 29 days; vincristine 1.5 mg/m^2 weekly for 4 weeks; daunomycin 25 mg/m^2 i.v. days 1 and 8; and asparaginase 10 000 IU/ m^2 i.m. 3 times per week $\times$ 9 doses
Weeks 5–6	Etoposide 300 mg/m^2 i.v. + cytarabine 300 mg/m^2 i.v. days 22, 25, 29
Weeks 7–8	Methotrexate 2 g/m^2 i.v. days 44 and 51; mercaptopurine 75 mg/m^2 p.o. daily
Continuation (120 weeks)	
Week 1	Etoposide 300 mg/m^2 i.v. + cyclophosphamide 300 mg/m^2 i.v.
Week 2	Mercaptopurine 75 mg/m^2 p.o. daily + methotrexate 40 mg/m^2 i.v./i.m.
Week 3	Methotrexate 40 mg/m^2 i.v./i.m. + cytarabine 300 mg/m^2 i.v.
Week 4	Prednisone 40 mg/m^2 p.o. daily + vincristine 1.5 mg/m^2 i.v. + asparaginase 10 000 IU/ m^2 i.m.
Week 5	Etoposide 300 mg/m^2 i.v. + cyclophosphamide 300 mg/m^2 i.v.
Week 6	Mercaptopurine 75 mg/m^2 p.o. + methotrexate 2 g/m^2 i.v.
Week 7	Etoposide 300 mg/m^2 i.v. + cytarabine 300 mg/m^2 i.v.
Week 8	Prednisone 40 mg/m^2 p.o. daily + vincristine 1.5 mg/m^2 i.v. + asparaginase 10 000 IU/ m^2 i.m.

Weeks 1–8 were repeated for a total of 120 weeks of therapy. At weeks 32–38, reinduction therapy was given which was identical to remission induction therapy, except that etoposide/cytarabine was given on day 22 only, and only one high-dose methotrexate was given. High-dose methotrexate was replaced by methotrexate 40 mg/m^2 after 1 year of continuation therapy, and asparaginase was omitted after reinduction.

Thus, asparaginase was administered for a total of 9 doses (on days 2, 4, 6, 8, 10, 12, 15, 17 and 19) during induction therapy and on the same days of reinduction (weeks 32–34). Asparaginase was also administered every 4 weeks during continuation therapy (weeks 4, 8, 12, 16, 20, 24 and 28).

Results

Patient characteristics are summarized in Table 2. One reacting patient (patient No. 7) did not receive asparaginase during induction therapy because of coagulation abnormalities, and thus received the first dose of asparaginase during the continuation phase. This patient experienced an initial reaction to *E. coli* asparaginase after receiving the fewest number of asparaginase doses, after the fourth dose in continuation therapy. Another reacting patient (patient No. 10) received asparaginase only during induction and reinduction phases due to development of hemorrhagic cerebral infarction during continuation unrelated to asparaginase therapy.

All allergic reactions occurred during continuation or reinduction phases, with initial reactions developing after 4–20 doses (median 11.5) of *E. coli* asparaginase. The first allergic reactions occurred with the fourth *E. coli* asparaginase dose ($n=1$), the 10th dose ($n=4$), the 11th dose ($n=6$), the 12th dose ($n=5$), the 13th dose ($n=3$), the 14th dose ($n=2$), and the 20th dose ($n=1$).

Characteristics of the allergic reactions experienced in the 22 patients are summarized in Table 3. The most frequent initial local manifestations at the site of injection were pain (nine cases), swelling (six cases), and erythema (five cases). Other local symptoms included urticaria and pruritis (one case each). The most frequent initial systemic manifestations were urticaria (seven cases), angioedema (six cases), erythema (five cases), pruritis (four cases), and fever (three cases). Other symptoms observed as part of the systemic reactions included wheezing (two cases), respiratory distress, and lower back pain (one case each). Clinical manifestations of subsequent reactions to either *E. coli* or *Erwinia* asparaginases and therapeutic interventions following any allergic reaction are presented in Table 3. Following a reaction to asparaginase, patients either continued on the same preparation with premedication or switched to another asparaginase product, as depicted in Figure 1. After the initial reaction to *E. coli* asparaginase, about 50% of the patients continued to receive the same preparation (with diphenhydramine or corticosteroid premedication) and the remainder of the patients were switched to *Erwinia* asparaginase. However, 10 of the 12 patients who continued on *E. coli* asparaginase eventually reacted again, and were subsequently switched to the *Erwinia* preparation. Eventually, 21 patients were able to tolerate asparaginase (with or without premedication), as depicted in Figure 1. One patient exhibited a clinical allergic reaction to

all three asparaginase preparations (*E. coli*, *Erwinia*, and PEG) and had the highest anti-asparaginase antibody concentration after reinduction and the greatest change in antibody level from post-induction to post-reinduction (from 0.001 to 1.841).

Clinical hypersensitivity reactions following *Erwinia* asparaginase occurred in five of 20 patients (25%) after a median of four doses (range 1–7). One patient reacted to the first *Erwinia* asparaginase dose, one to the third dose, two to the fourth dose, and one to the seventh dose. All nonreacting patients received 25 doses of *E. coli* asparaginase, whereas reacting patients received a median of 14 doses of *E. coli* asparaginase (range 10–25) and a median of 25 doses (range 15–26) of either *E. coli* or *Erwinia* asparaginase.

Figure 2 depicts anti-asparaginase antibody concentrations post-induction and post-reinduction in reacting and nonreacting patients. In the 22 patients who developed clinical hypersensitivity, the median post-induction anti-asparaginase antibody concentration (ie before any allergic reaction) was 0.039 (range 0.001–0.375), whereas the median post-reinduction (ie after hypersensitivity reactions) anti-asparaginase antibody level was 0.506 (range 0.008–1.84, $P=0.0002$). In the 13 patients who did not exhibit clinical hypersensitivity, median post-induction level (ie after nine asparaginase doses) increased from 0.011 (range 0.004–0.064) to 0.032 (range 0.004–1.56) at post-reinduction (ie after 25 doses of asparaginase) ($P=0.02$). Interestingly, both median post-induction and post-reinduction anti-asparaginase antibody levels were higher in reacting than in nonreacting patients ($P=0.004$ and $P=0.01$, respectively). After induction and reinduction, 59 and 91% of reacting patients, respectively, had anti-asparaginase concentrations greater than three-fold the background levels of plasma from untreated human volunteers, whereas 15 and 54%, respectively, of nonreacting patients exceeded this threshold at identical time points. Anti-asparaginase antibody levels post-induction in nonreacting patients were not significantly different from those of untreated normal human volunteers ($P=0.985$). Anti-asparaginase antibody concentrations increased from post-induction to post-reinduction in 20 of 22 reacting and in eight of 13 nonreacting patients ($P=0.08$, χ^2). Antibody levels were inversely related to the elapsed time between the occurrence of the last clinical reaction and sampling ($P=0.011$) (Figure 3).

Discussion

This is the first report of anti-asparaginase antibody levels at fixed time points relative to conventional three times weekly intramuscular *E. coli* asparaginase treatment in patients with newly diagnosed ALL. These data are important because repeated administration, time of treatment, development of relapse, and concurrent chemotherapy have all been suggested to influence antibody levels and/or allergic reactions;^{4,12,14–17} thus, it is useful to have data in which all of these variables have been uniform. We found that anti-asparaginase antibodies increased after a fixed period of therapy in the majority of patients, regardless of whether they developed hypersensitivity reactions. However, antibody levels were higher among patients who developed allergic reactions than in those who did not, and they were higher both before and after the occurrence of hypersensitivity in such patients. Antibody analysis may be of clinical value, as elevated antibody levels were detected before reactions, and thus they may predict future hypersensitivity. This may be useful if patients are

Table 2 Patient characteristics according to occurrence of allergic reactions

Characteristic	Reacting patients	Nonreacting patients
No. of patients	22	13
Sex		
Male	6	5
Female	16	8
Median age (years)	4.8	3.3
Range	1.0–17.2	2.4–15.1
Race		
White	16	11
Black	3	2
Hispanic	3	0
Immunophenotype		
B lineage	22	11
T lineage	0	2

Table 3 Summary of patient reactions to asparaginase

Patient No.	Asparaginase preparation	No. of doses ^a	Description of reaction ^b	Therapeutic intervention ^c
1	(a) <i>E. coli</i>	11	(a) erythema, pruritis, urticaria, angioedema, wheezing	(a) diphenhydramine, hydrocortisone, epinephrine, hydroxyzine
2	(b) <i>Erwinia</i>	14	(b) none	(b) none
	(a) <i>E. coli</i>	11	(a) pruritis, urticaria, angioedema, wheezing	(a) diphenhydramine, epinephrine, breathing treatment, prednisone
	(b) <i>Erwinia</i>	3	(b) erythema, pruritis, urticaria, angioedema	(b) none
	(c) <i>Erwinia</i> ^d	11	(c) none	(c) none
3	(a) <i>E. coli</i>	10	(a) erythema, urticaria, angioedema	(a) diphenhydramine, epinephrine, hydrocortisone
	(b) <i>Erwinia</i>	7	(b) swelling, pain	(b) diphenhydramine, cold pack
	(c) <i>Erwinia</i> ^d	3	(c) erythema, angioedema	(c) diphenhydramine
	(d) PEG	1	(d) erythema, angioedema	(d) diphenhydramine
4	(a) <i>E. coli</i>	12	(a) swelling, pain	(a) hot pack, cold pack, acetaminophen
	(b) <i>E. coli</i> ^d	4	(b) pain	(b) acetaminophen, diphenhydramine, cold pack
	(c) <i>Erwinia</i>	9	(c) none	(c) none
5	(a) <i>E. coli</i>	10	(a) erythema	(a) diphenhydramine
	(b) <i>E. coli</i> ^d	2	(b) erythema, urticaria	(b) none
	(c) <i>Erwinia</i>	13	(c) none	(c) none
6	(a) <i>E. coli</i>	12	(a) fever, pain	(a) diphenhydramine
	(b) <i>Erwinia</i>	13	(b) none	(b) none
7	(a) <i>E. coli</i>	4	(a) erythema, swelling	(a) none
	(b) <i>E. coli</i> ^d	6	(b) angioedema	(b) diphenhydramine
	(c) <i>Erwinia</i>	5	(c) none	(c) none
8	(a) <i>E. coli</i>	10	(a) pruritis, urticaria	(a) diphenhydramine, hydrocortisone
	(b) <i>Erwinia</i>	14	(b) none	(b) none
9	(a) <i>E. coli</i>	13	(a) swelling, pain	(a) diphenhydramine
	(b) <i>Erwinia</i>	8	(b) none	(b) none
10	(a) <i>E. coli</i>	12	(a) pain	(a) none
	(b) <i>E. coli</i> ^d	2	(b) pain	(b) codeine
	(c) <i>Erwinia</i>	4	(c) none	(c) none
11	(a) <i>E. coli</i>	12	(a) swelling, pain	(a) none
	(b) <i>Erwinia</i>	4	(b) none	(b) none
	(c) <i>E. coli</i>	2	(c) erythema	(c) cephalixin, diphenhydramine
	(d) <i>E. coli</i>	1	(d) swelling, pain	(d) cephalixin, diphenhydramine
	(e) <i>Erwinia</i>	4	(e) erythema, swelling, pain	(e) none
	(f) <i>Erwinia</i>	2	(f) none	(f) none
12	(a) <i>E. coli</i>	10	(a) erythema, urticaria, angioedema	(a) heat pack
	(b) <i>E. coli</i> ^d	6	(b) erythema, vomiting, cyanosis, respiratory distress, angioedema	(b) cold pack, O ₂ , epinephrine
	(c) <i>Erwinia</i>	9	(c) none	(c) none
13	(a) <i>E. coli</i>	14	(a) erythema, pruritis	(a) diphenhydramine
	(b) <i>Erwinia</i>	1	(b) erythema	(b) none
	(c) <i>Erwinia</i> ^d	10	(c) none	(c) none
14	(a) <i>E. coli</i>	12	(a) swelling, pain	(a) diphenhydramine, meperidine, acetaminophen + codeine, warm pack
	(b) <i>E. coli</i> ^d	4	(b) swelling, pain	(b) acetaminophen + codeine
	(c) <i>Erwinia</i>	4	(c) pain	(c) warm pack, acetaminophen + codeine
	(d) <i>Erwinia</i>	5	(d) none	(d) none
15	(a) <i>E. coli</i>	13	(a) fever, pain	(a) vancomycin, ceftazidime
	(b) <i>E. coli</i> ^d	1	(b) erythema, swelling	(b) none
	(c) <i>Erwinia</i>	11	(c) none	(c) none
16	(a) <i>E. coli</i>	11	(a) urticaria	(a) none
	(b) <i>E. coli</i> ^d	6	(b) urticaria, fever, pain	(b) acetaminophen
	(c) <i>Erwinia</i> ^d	8	(c) none	(c) none
17	(a) <i>E. coli</i>	11	(a) fever, erythema, urticaria, angioedema	(a) diphenhydramine
	(b) <i>E. coli</i> ^d	8	(b) erythema, angioedema	(b) diphenhydramine
	(c) <i>Erwinia</i>	6	(c) none	(c) none
18	(a) <i>E. coli</i>	13	(a) erythema, angioedema, respiratory distress	(a) O ₂ , diphenhydramine, hydrocortisone, epinephrine
	(b) <i>Erwinia</i>	8	(b) none	(b) none
19	(a) <i>E. coli</i>	14	(a) erythema, pain	(a) none
	(b) <i>E. coli</i> ^d	1	(b) swelling, pain	(b) diphenhydramine
	(c) <i>Erwinia</i>	11	(c) none	(c) none
20	(a) <i>E. coli</i>	11	(a) erythema, pruritis	(a) diphenhydramine
	(b) <i>Erwinia</i>	14	(b) none	(b) none
21	(a) <i>E. coli</i>	20	(a) swelling, pain	(a) diphenhydramine, meperidine, lorazepam, acetaminophen + codeine
	(b) <i>E. coli</i> ^d	5	(b) none	(b) none
22	(a) <i>E. coli</i>	11	(a) urticaria	(a) diphenhydramine
	(b) <i>E. coli</i> ^d	14	(b) none	(b) none

^aThe number of doses of the indicated asparaginase preparation given prior to and including the dose associated with the reaction depicted in the next column.

^bClinical characteristics of each reaction occurring after the indicated number of doses of the specified asparaginase preparation.

^cInterventions taken to treat each reaction described in the previous column.

^dPatients received premedication of diphenhydramine with or without corticosteroids before asparaginase doses.

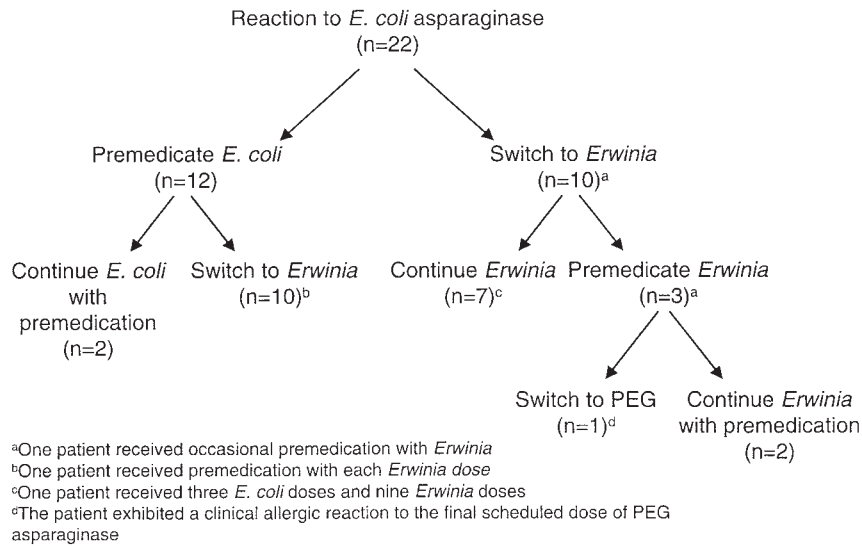


Figure 1 Schematic of asparaginase therapy in patients exhibiting clinical hypersensitivity reactions ($n = 22$). Twenty-one patients were able to tolerate asparaginase (with or without premedication); one patient reacted to all three asparaginase preparations.

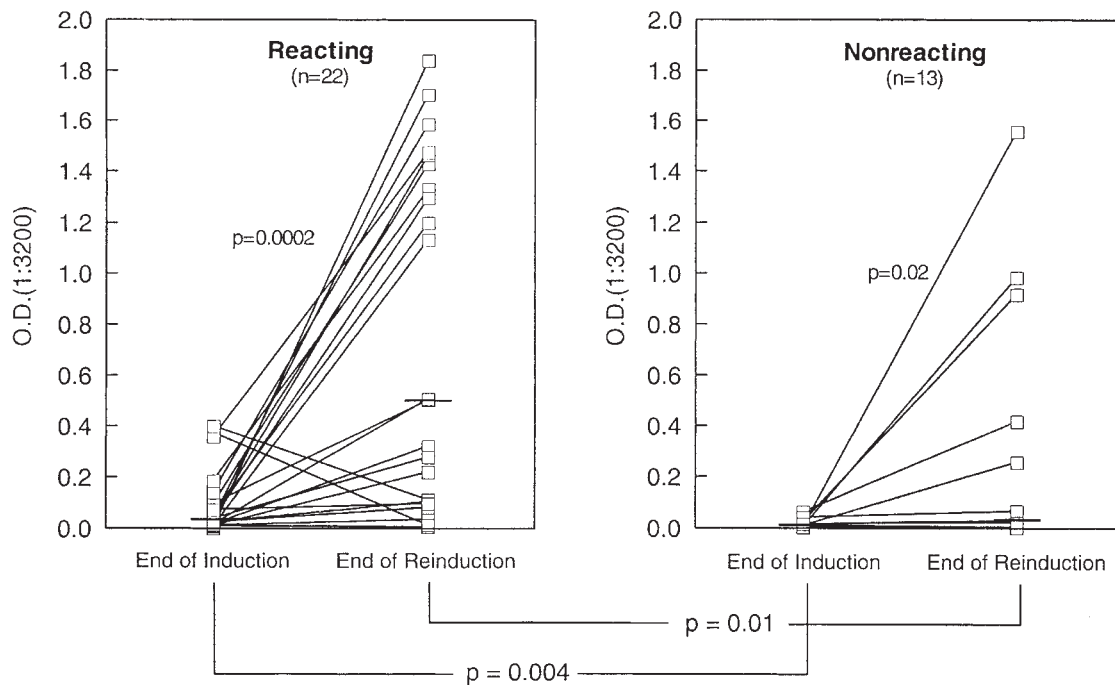


Figure 2 Anti-asparaginase antibody concentrations measured at the end of induction (after a median of nine doses of asparaginase and before any reactions) and at the end of reinduction (after a median of 25 doses and after at least one allergic reaction) in patients who did and did not develop hypersensitivity reactions. Thick, solid lines represent the median antibody level.

to receive multiple or additional courses of asparaginase treatment.

The reported incidence of patients demonstrating hypersensitivity reactions to *E. coli* asparaginase has been widely divergent, varying from 0 to 45% of treated patients. One difficulty in interpreting incidences of reactions is that a number of preparations of *E. coli* asparaginase prepared by different manufacturers have been used by various treatment groups. Because native *E. coli* asparaginase prepared by different manufacturers may vary markedly in their pharmacokinetic and pharmacodynamic properties,¹⁸ it is important to indicate

the specific commercial product used in clinical trials, which has not been done uniformly in the past.

Several other variables may also influence the incidence of allergic reactions to asparaginase, and thus may explain the variability in the reported frequency. The risk of hypersensitivity reactions may increase with continuing administration of asparaginase or with readministration following a period of no asparaginase therapy of at least 1 month.⁴ A number of different dosages and schedules have been employed, but whether the incidence of allergic reactions is dose- or schedule-dependent is controversial. Oettgen *et al*¹² reported that the risk of

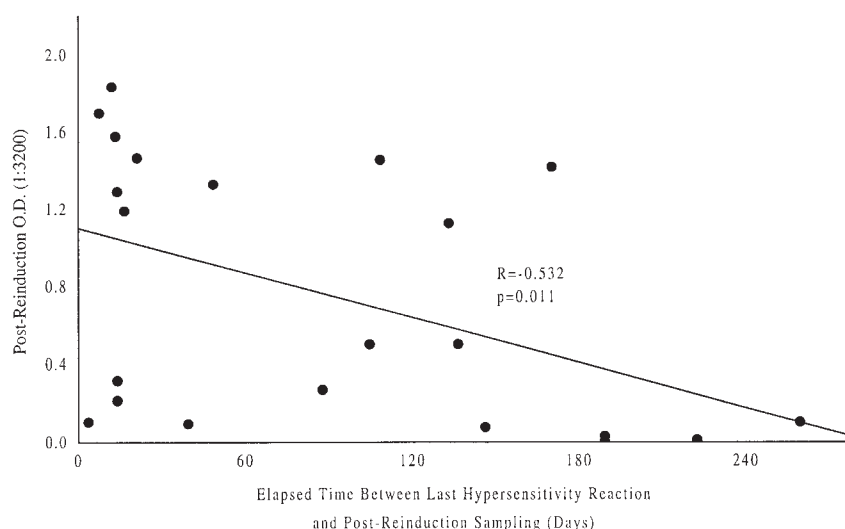


Figure 3 Relationship between anti-asparaginase antibody levels measured at the end of reinduction (after a median of 25 doses and allergic reactions) and elapsed time between the last reaction and sample collection for post-reinduction antibody levels.

reactions is dose-related, whereas Jaffe *et al*¹⁹ suggested that the risk is independent of dose or schedule. Concomitant administration with other chemotherapeutic agents, such as mercaptopurine,¹⁶ cyclophosphamide¹⁴ and prednisone-vincristine^{4,15,17} was associated with a reduced incidence of allergic reactions or antibody response in some studies, but not in others.^{8,19} Because all patients in our study were never previously exposed to asparaginase, received identical chemotherapy, and were studied at uniform times, previous or varying concurrent chemotherapy are not confounding factors in our analysis.

We found that 25% of patients who reacted to *E. coli* asparaginase eventually reacted to *Erwinia* asparaginase, similar to or slightly higher than previous reports.^{4,5,9,15,20} All reacting patients, except for one, reacted to *E. coli* asparaginase after 10 doses, but those who subsequently reacted to *Erwinia* asparaginase reacted shortly thereafter, within seven doses. Evans *et al*⁴ observed that the risk of anaphylactoid reactions to *Erwinia* asparaginase was significantly greater in patients who had previously reacted to *E. coli* asparaginase, and that the risk of a reaction was related to the total number of asparaginase doses given. This was supported by Dellinger *et al*,⁸ who observed that patients receiving more than four courses of asparaginase were susceptible to anaphylaxis, with a 32% risk of reaction with each dose.

The presence of high antibody levels post-induction, but prior to development of allergy, was associated with subsequent allergic reactions. Seventy-seven percent of patients who developed an allergic reaction during continuation or reinduction treatment had a post-induction antibody concentration greater than 0.17, whereas only 25% of nonreacting patients exceeded this level post-induction. Median anti-asparaginase antibody levels after induction in nonreacting patients were not significantly different from those of untreated normal human volunteers. Thus, patients with an antibody level greater than 0.17 after nine doses of intramuscular *E. coli* asparaginase during induction may be at higher risk of developing a hypersensitivity reaction during continuation or reinduction therapy. Although post-induction and post-reinduction antibody levels were both lower in nonreacting patients compared to reacting patients, anti-asparaginase antibody concentrations, interestingly, increased follow-

ing multiple asparaginase doses, regardless of whether clinical hypersensitivity was exhibited (median of 13-fold increase in reacting patients vs three-fold in nonreacting patients). However, there was no relationship between severity of allergic reaction and antibody level.

Development of hypersensitivity reaction and successive exposure to asparaginase may attenuate the pharmacologic effect of asparaginase.^{3,21} Serum asparaginase activity was undetectable in four of five patients analyzed within 1 week of an anaphylactic reaction to *E. coli* asparaginase.³ Also, the half-life of PEG asparaginase was shorter in five patients with a history of an allergic reaction to *E. coli* asparaginase than in patients who had not had prior allergic reactions.³ In addition, asparagine plasma depletion was observed in 80% of patients during the first exposure to *Erwinia* asparaginase and in only 25% of patients during the second or third exposures.²¹ However, clinical studies documenting an adverse effect of sub-clinical or clinical allergy to asparaginase on antileukemic outcome are lacking. At this point, the best course of action after asparaginase allergy is not clear. Our current protocol specifies switching from the *E. coli* to the *Erwinia* preparation as soon as an allergic reaction occurs because, as described here, premedication did not prevent subsequent reactions in the majority of cases (10 of 12 patients who received premedicated *E. coli* asparaginase following an initial reaction developed a subsequent reaction to this preparation); patients with allergy to *Erwinia* are then switched to PEG asparaginase.

We found that development of anti-asparaginase antibodies following *E. coli* asparaginase is common in children with ALL, is higher in those who develop allergy (even prior to clinical reaction), and increases following repeated dosing. High antibody concentrations post-induction may be useful in predicting which patient will develop allergic reactions subsequently. In such patients, alternative forms of asparaginase, such as PEG or *Erwinia* preparations, may be needed.

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