Antibody Against Poly(Ethylene Glycol) Adversely Affects PEG-Asparaginase Therapy in Acute Lymphoblastic Leukemia Patients

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BACKGROUND. Rapid clearance of poly(ethylene glycol)-asparaginase (PEG-ASNase) has been reported for up to one-third of patients treated for acute lymphoblastic leukemia (ALL), potentially rendering their treatment ineffective. A 25% occurrence of an antibody against PEG (anti-PEG) was previously reported in healthy blood donors. The objective of the study was to determine whether anti-PEG was associated with rapid clearance PEG-ASNase.

METHODS. The investigation reanalyzed stored sera from pediatric patients enrolled in the ALL Berlin-Frankfurt-Muenster 2000 studies. Twenty-eight samples were selected to include 15 subjects with undetectable ASNase activity after receiving PEG-ASNase. Sixteen subjects treated with unmodified ASNase were also included, 8 with low ASNase activity. Sera were tested for anti-PEG using 2 techniques: 1) serology, by agglutination of PEG-coated red blood cells; 2) flow cytometry, by analysis of 10 μm PEG beads stained for bound immunoglobulins.

RESULTS. Of the 15 sera from PEG-ASNase-treated patients with undetectable ASNase activity, anti-PEG was detected in 9 by serology and in 12 by flow cytometry. Anti-PEG was detected in 1 PEG-ASNase-treated patient with lower ASNase activity (123 U/L). No relation was observed between anti-PEG and serum ASNase activity for patients treated with unmodified ASNase.

CONCLUSIONS. The presence of anti-PEG was very closely associated with rapid clearance of PEG-ASNase. Further comprehensive studies are warranted to fully elucidate the effect of anti-PEG on PEG-conjugated agents. Screening and monitoring for anti-PEG may allow identification of patients for whom a modified dosing strategy or use of a non-PEGylated drug would be appropriate. ***Cancer* 2007;110:103–11.** © *2007 American Cancer Society.*

KEYWORDS: acute lymphoblastic leukemia, antibody, poly(ethylene glycol), asparaginase medac, PEG-asparaginase, Oncaspar.

Immunogenicity, antigenicity, toxicity, and rapid renal clearance of exogenous therapeutic agents are critical concerns for the pharmaceutical industry. Altering the properties of agents to suppress drug clearance and reduce toxicity while maintaining potency and efficacy are therefore goals for the introduction of new therapies. One very successful and widely used method to reduce clearance and toxicity is the covalent attachment of poly(ethylene glycol) (PEG) to the therapeutic agent.[1–21] This approach 'masks' the agent from the host's immune system, reduces toxicity, and slows renal clearance by increasing the hydrodynamic size of the agent.

Modification of exogenous proteins by the covalent attachment of PEG was first reported in 1977 by Abuchowski et al.[1,2] They demonstrated a significant increase in the circulatory time and that

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immunogenicity and antigenicity was almost abolished for PEGylated proteins versus the unmodified protein. The covalent attachment of PEG to a wide range of therapeutic agents is now commonly used, including PEG-asparaginase (PEG-ASNase, [Pegaspargase, Oncaspar]) for leukemia and lymphoma,[3] PEG-interferon alpha 2a (Pegasus) and 2b (PEG-Intron) for chronic hepatitis C virus,[4,5] PEG-filgrastim (Neulasta) for neutropenia,[6,7] and PEG-adenosine deaminase for severe combined immunodeficiency syndrome.[8] In addition, numerous other agents for PEGylation are in use or are being developed.[9–21]

Whereas PEG has long been claimed to be nonantigenic and weakly immunogenic,[9–13] the existence of a naturally occurring antibody against PEG (anti-PEG) has been known since 1983,[22] and the antibody has been successfully induced in animal models.[23–27] Richter and Akerblom[22] determined that the occurrence of anti-PEG in the healthy population was 0.2%, and from their studies with allergic patients determined the anti-PEG to be predominantly immunoglobulin M (IgM). They concluded that the antibody was of no clinical significance for PEG-modified allergens in hyposensitization therapy.[22] More recently, we detected a markedly higher occurrence (22%–25%) of anti-PEG in the healthy blood donor population (350 donors) and identified both IgG and IgM.[28–30] Anti-PEG was induced in 5 of 13 patients treated for chronic refractory gout with PEG-uricase, identified as a low titer IgG and IgM. Rapid clearance of PEG-uricase was observed in this subset of patients, limiting the potential use of this agent for treatment of hyperuricemia.[31]

Rapid clearance of PEG-ASNase has been observed for a subgroup of pediatric patients treated for acute lymphoblastic leukemia (ALL)[32,33] without any clinical evidence of an allergic reaction. The rapid clearance of PEG-ASNase may well render the treatment ineffective, as it is commonly agreed that a trough serum ASNase activity of $\geq$100 U/L for 14 days and $\geq$50 U/L for 21 days is required to deplete asparagine (ASN) to achieve a complete response in ALL.[34,35]

The present study was primarily designed to determine whether the presence of anti-PEG was associated with rapid clearance of PEG-ASNase observed for a subgroup of pediatric patients treated for ALL,[32,33] with a secondary goal of establishing a simple and reliable technique for the detection of anti-PEG in blood sera.

MATERIALS AND METHODS

Serum sampling was performed during the Acute Lymphoblastic Leukemia Berlin-Frankfurt-Muenster (ALL-BFM) 2000 studies, which are multicenter therapy optimization studies for the treatment of ALL in children.[36,37] The protocol was approved by local ethics committees and was carried out according to the Declaration of Helsinki. Informed consent was obtained from patients and/or their parents for blood sampling, drug monitoring, and research when entering the study.

Patients

This study reanalyzed stored serum samples collected from 44 pediatric leukemia patients who were enrolled in the ALL-BFM 2000 and the ALL-BFM REZ 2002 study. Twenty-eight received PEG-ASNase (Oncaspar, Wedel, Germany; 1000 U/m^2, 17 male, 11 female, mean age = 8.3 years), and 16 received unmodified *Escherichia coli* ASNase (Medac; 5000 or 10,000 U/m^2, 10 male, 6 female, mean age 8.8 years). There was no significant difference in age between the 2 treatment groups ($P = .761$). Patients in the PEG-ASNase group had received up to 8 doses of *E. coli* ASNase or up to 5 doses of *Erwinia chrysanthemi* ASNase. Recurrence patients from the ALL-BFM REZ 2002 study had received up to 6 previous doses of PEG-ASNase. Twelve patients in the PEG-ASNase group had no recorded prior exposure to any ASNase, 7 of which had undetectable ASNase activity. Nine patients in the PEG-ASNase group were enrolled in the ALL-BFM REZ 2002 study, and 19 patients were enrolled in the ALL-BFM 2000 study. After enrollment in the studies, serum samples were typically collected after the first dose of PEG-ASNase.

Patients in the unmodified ASNase group had received up to 8 doses of ASNase before sampling. No patients in this group had any documented prior exposure to PEG-ASNase or *Erwinia* ASNase. All patients in the ASNase group were enrolled in the ALL-BFM 2000 study. Typically, serum samples were collected after the seventh dose and before administration of the eighth dose of ASNase.

Serum samples were collected at 0 to 15 days after drug administration (mean = 7.1 days for PEG-ASNase and 3.0 days for ASNase) and were assayed for ASNase activity using a microplate technique.[38] Note that the 28 serum samples from PEG-ASNase-treated patients were selected to include 15 subjects with undetectable ASNase activity and 13 with effective ASNase activity (>100 U/L).[34,35] Similarly, the serum samples from 16 subjects treated with unmodified ASNase were selected to include 8 subjects with ASNase below therapeutic levels (<50 U/L) activity and 8 with effective ASNase activity (>50 U/L).[34,35]

Testing for Anti-PEG

All testing for anti-PEG was performed in a blinded manner with samples identifiable only by means of a code number. Data for ASNase activity and treatment group were integrated with serological and flow cytometric results only after all anti-PEG testing was completed.

Preparation of Reagent PEG-RBCs

Blood group O RBCs (red blood cells) were washed with 10 mM Dulbecco phosphate buffered saline ([PBS] pH 7.4, 290 mOsmol/kg), then resuspended at 10% hematocrit in 15 mM triethanolamine buffer (pH 8.2, 290 mOsmol/kg). A fresh solution of methoxy-poly(ethylene glycol)-succinimidyl propionate of molecular mass 20 kDa (Nektar Therapeutics, Huntsville, Ala) dissolved in cold 10 mM HCl/saline was added to the RBC suspension to yield a final polymer concentration of 10 mg/mL. The sample was incubated at room temperature for 1 hour and then washed 3 times with PBS.[29] PEG-RBCs and control RBCs (without PEG) were resuspended to a final hematocrit of 5% in PBS.

Anti-PEG by Serology

Two drops of test serum were added to 1 drop of control or PEG-coated 5% hematocrit RBC suspensions in a 10 × 75 mm glass test tube, mixed gently, and incubated at room temperature for 15 minutes. The suspension was centrifuged at 500 *g* for 1 minute and agglutination scored according to the 0–4+ scale.[39] Samples that were positive for anti-PEG caused agglutination of the PEG-coated RBCs but not the control RBCs.

Anti-PEG by Flow Cytometry

A 1% (w/v) stock suspension of 10-μm diameter TentaGel-OH particles (RAPP Polymere, Tubingen, Germany) was prepared in PBS and briefly sonicated to disrupt any particulate aggregates. Twenty-five microliters of the TentaGel-OH stock was added to 50 μL of serum and 100 μL of PBS, the sample was incubated at room temperature for 30 minutes, and then washed 3 times with PBS (200 *g* for 2 minutes). The centrifuged pellet was resuspended in 0.5 mL of PBS containing 5 μL of fluorescein-labeled antihuman IgG (whole molecule goat FITC-anti-IgG, Sigma Chemical Co., St. Louis, Mo) and 2.5 μL of R-phycoerythrin-labeled antihuman IgM (μ-chain specific goat RPE-anti-IgM, Sigma), mixed gently, and then incubated in the dark for 1 hour at room temperature. The particles were washed (3 times, PBS, 200 *g* for 2 minutes), resuspended in PBS, and the mean fluorescence intensity of 10,000 single TentaGel-OH particles was determined by flow cytometry (EPICS-XL MCS, Becton Dickinson Biosciences, San Jose, Calif; FL-1 and FL-2 channels for FITC and RPE, respectively). Samples with a mean fluorescence intensity of >100 for IgG and/or >50 for IgM were identified as positive for anti-PEG.

Testing for Anti-ASNase

L-ASNase (Sigma) was dissolved in Immunopure Binding Buffer (Pierce Biotechnology, Rockford, Ill), pipetted into a 96-well black plate at a protein concentration of 50 μg/well (10 U/well) and incubated for 4 hours at room temperature. The wells were washed (5×) with PBS, blocked overnight at 4°C with 4% milk/2% bovine serum albumin (BSA), then washed again (5×) with PBS. Five μL of serum + 150 μL of binding buffer were added to each well, incubated for 2 hours at room temperature, and PBS-washed 5 times. One hundred microliters of PBS containing 5 μL of FITC-antihuman IgG and 2.5 μL of RPE-antihuman IgM were added, incubated for 1 hour at room temperature, and then washed (5×) with PBS. Two hundred microliters of PBS were added and fluorescence recorded using a microplate reader (GENios Pro, Tecan Systems, San Jose, Calif) with absorbance/emission filters of 485/535 nm and 485/595 nm for IgG and IgM, respectively. Anti-ASNase positives showed higher fluorescence than uncoated control wells after correction for background fluorescence.

Statistical Analysis

Unless otherwise stated, differences between groups were tested using the Student *t*-test. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of flow cytometric and serological determination of anti-PEG were derived using the ASNase activity level as the gold standard. The 95% confidence intervals (CI) were derived using the binomial and F probability distributions.

RESULTS

The mean serum ASNase activities for all samples tested were 240 U/L for the PEG-ASNase group and 84 U/L ASNase group.

By serological testing with PEG-RBCs, 9 of the 28 sera (32%) from patients treated with PEG-ASNase contained anti-PEG (Fig. 1). Strong agglutination (4+) was noted for 6 patients, whereas 3 showed weaker but still clearly positive agglutination (2+w – 2+). The ASNase activity in all 9 of the anti-PEG-positive sera was below the limit of detection (<5 U/L).

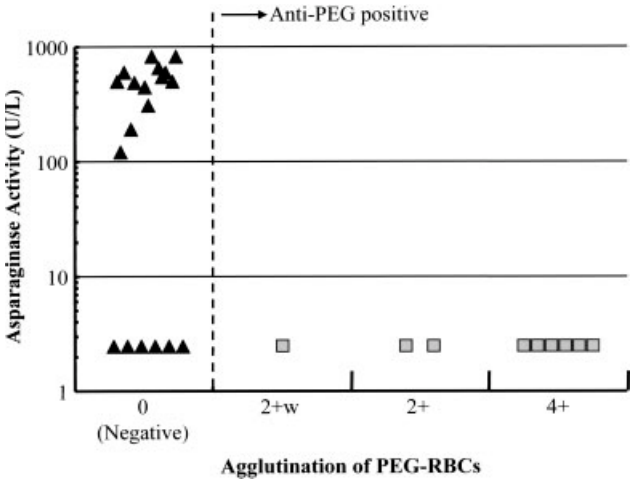

FIGURE 1. Serological identification of anti-PEG (poly[ethylene glycol]) versus asparaginase activity for PEG-asparaginase treated patients. Anti-PEG positive patients (squares, n = 9) show undetectable asparaginase activity, whereas anti-PEG negative patients (triangles, n = 19) show a mean asparaginase activity of 353 U/L.

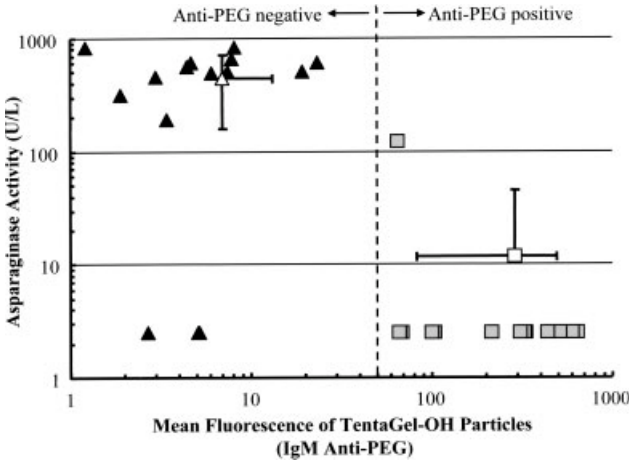

FIGURE 2. Flow cytometric identification of IgM anti-PEG (poly[ethylene glycol]) versus asparaginase activity for PEG-asparaginase-treated patients. Anti-PEG-positive patients (squares, n = 13) show negligible asparaginase activity for 12 sera and 1 sample with low asparaginase activity (123 U/L) with an overall mean activity of 12 U/L. Anti-PEG negative patients (triangles, n = 15) show a high asparaginase activity (mean = 438 U/L). Mean data are shown with open symbols ± SD ($P = 3.6 \times 10^{-5}$).

By using the flow cytometry-based assay, sera from 13 (46%) of the PEG-ASNase treated patients showed a positive signal for IgM anti-PEG (Fig. 2), 12 of which showed undetectable ASNase activity (<5 U/L), 1 sample had an ASNase activity of 123 U/L, at the low end of the therapeutic range. All 9 patients who were positive for anti-PEG by serology were positive for IgG and IgM anti-PEG by flow cytometry (IgG data not shown); the remaining 4 patients with a positive result by flow cytometry only showed relatively weak fluorescence (<100) for IgM and were negative for IgG. Both serological and flow cytometric analyses showed that all anti-PEG-positive patient sera treated with PEG-ASNase showed low or negligible ASNase activity: 1) <5 U/L versus a mean of 353 U/L for anti-PEG-positive versus negative via serology, $P = 7.7 \times 10^{-5}$; 2) means of 12 and 438 U/L for anti-PEG-positive versus negative via flow cytometry, $P = 3.6 \times 10^{-5}$.

Of the 12 patients in the PEG-ASNase group that had no documented prior exposure to PEG-ASNase, 7 (58%) had undetectable ASNase activity, 4 (33%) of which were positive for anti-PEG. Of the 16 patients in the PEG-ASNase group with documented prior exposure to PEG-ASNase, 8 (50%) had undetectable ASNase activity, all of which were positive for anti-PEG. Nine (32%) of 28 patients in the PEG-ASNase group had documented prior exposure to unmodified ASNase, 6 (21%) had undetectable ASNase activity and were also positive for anti-PEG.

Four sera from PEG-ASNase patients yielded somewhat unusual results: 1 weak IgM anti-PEG-positive sample showed measurable ASNase activity and 3 anti-PEG negative patients had undetectable ASNase activity, 2 of which were positive for anti-ASNase. Table 1 presents the measures of accuracy and predictability of the flow cytometry and serological methods of anti-PEG determination for ASNase activity in patients treated with PEG-ASNase. The positive/negative predictive values of anti-PEG for ASNase activity were 92%/80% and 100%/68% for flow cytometric and serological detection of anti-PEG. The overall accuracy was 86% (95% CI: 37%, 96%) for flow cytometry determinations and 79% (95% CI: 59%, 92%) for serological determinations.

For the patients treated with unmodified ASNase (Medac), 2 of 16 sera (13%) contained anti-PEG by serology (Fig. 3): 1 with ASNase 9activity sufficient for asparagine depletion (135 U/L) and 1 with low ASNase activity (49 U/L). Six of 16 sera (38%) were identified as anti-PEG-positive by flow cytometry (Fig. 4), 3 with ASNase activity >100 U/L and 3 with ASNase activity <50 U/L. No association was observed between serum ASNase activity and anti-PEG (means of 82 and 85 U/L for anti-PEG-positive versus negative by flow cytometry, respectively, $P = .947$). For ASNase-treated patients, the positive/negative predictive values of anti-PEG for ASNase activity were 50%/50% for both flow cytometric and serological detection of anti-PEG. The overall accuracy was 50% (95% CI: 25%, 75%) for both flow cytometry and serological determinations, and all

TABLE 1
Accuracy of the Identification of Anti-poly(ethylene glycol) (PEG) as a Predictor of Asparaginase Activity

	Flow cytometry determination in patients treated With PEG-asparaginase (oncaspar)		Serological determination in patients treated With PEG-asparaginase (oncaspar)		Flow cytometry determination in patients treated With asparaginase (medac)		Serological determination in patients treated With asparaginase (medac)	
Measure	Percentage (95% CI)	n/N	Percentage (95% CI)	n/N	Percentage (95% CI)	n/N	Percentage (95% CI)	n/N
Sensitivity for no/low asparaginase activity that are anti-PEG positive	80% (52%, 96%)	12/15	60% (32%, 84%)	9/15	38% (9%, 75%)	3/8	13% (0%, 53%)	1/8
Specificity for high asparaginase activity that are anti-PEG negative	92% (64%, 100%)	12/13	100% (75%, 100%)	13/13	63% (25%, 91%)	5/8	88% (47%, 100%)	7/8
Positive predictive value for anti-PEG to predict no/low asparaginase activity	92% (64%, 100%)	12/13	100% (66%, 100%)	9/9	50% (12%, 88%)	3/6	50% (1%, 99%)	1/2
Negative predictive value for anti-PEG to predict high asparaginase activity	80% (52% 96%)	12/15	68% (43%, 87%)	13/19	50% (19%, 81%)	5/10	50% (23%, 77%)	7/14
Overall accuracy	86% (67%, 96%)	24/28	79% (59%, 92%)	22/28	50% (25%, 75%)	8/16	50% (25%, 75%)	8/16

CI indicates confidence interval; n, numerator; N, denominator.

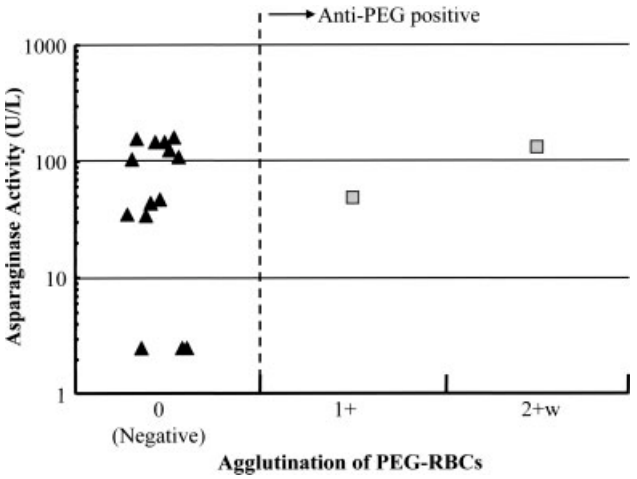

FIGURE 3. Serological identification of anti-PEG (poly[ethylene glycol]) versus asparaginase activity for asparaginase (Medac)-treated patients. Anti-PEG-positive patients (squares, n = 2) show 1 with normal and 1 with low asparaginase activity.

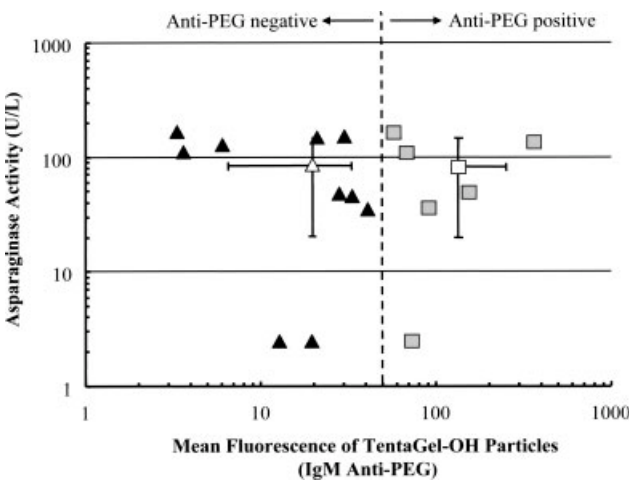

FIGURE 4. Flow cytometric identification of IgM anti-PEG (poly[ethylene glycol]) versus asparaginase activity for asparaginase (Medac)-treated patients. Anti-PEG positive patients (squares, n = 6) show 3 with low asparaginase activity and 3 with normal asparaginase activity (overall mean = 82.4 U/L). Anti-PEG-negative patients (triangles, n = 10) show 5 with low asparaginase activity and 5 with normal asparaginase activity (overall mean = 84.7 U/L).

measures are significantly lower than for patients treated with PEG-ASNase.

Twenty-two patient sera were tested for anti-ASNase (14 PEG-ASNase sera and 8 ASNase sera), 9 of which tested positive (data not shown). Seven of 14 PEG-ASNase-treated patient sera were positive for anti-ASNase: 4 were also positive for anti-PEG with undetectable ASNase activity, 1 was positive for anti-PEG with low ASNase activity (123 U/L), and 2 were negative for anti-PEG but had undetectable ASNase activity (<5 U/L). Seven of 14 PEG-ASNase-treated patient sera were negative for anti-ASNase: 5 were also positive for anti-PEG with undetectable ASNase activity, 2 were negative for anti-PEG (one with ASNase activity of 509 U/L and 1 with undetectable ASNase activity, <5 U/L).

No association was observed for ASNase activity versus anti-ASNase-positives for the PEG-ASNase treatment group ($P = .484$). Two of 8 patients tested

in the ASNase group were positive for anti-ASNase, 1 with undetectable ASNase activity and 1 with effective ASNase activity (152 U/L).

DISCUSSION

The use of ASNases as a frontline treatment in ALL is based on the findings that leukemic blast cells are dependent on the extracellular availability of ASN.[34,35,40–42] Depletion of ASN leads to impaired protein, DNA and RNA synthesis, and consequent cell death in malignant cells. To attain complete response to treatment of ALL with ASNase, a trough serum ASNase activity of $\geq$100 U/L for 14 days and $\geq$50 U/L for 21 days is recommended for serum and cerebrospinal fluid asparagine depletion.[34,35,42,43] Different ASNases (*E. coli, Erwinia,* and PEG-ASNase) have markedly different activities and clearances (half-lives from 16 hours for *Erwinia* to 7 days for PEG-ASNase)[34,35,40–42] and it is clear that monitoring of ASNase activity is an important factor for the effective treatment of ALL irrespective of allergic reactions, hypersensitivity, or silent inactivation.

The activity-time course after administration of PEG-ASNase is characterized by a low distribution volume and low clearance, resulting in clinically relevant activities for more than 3 weeks in most patients.[43] Despite the finding that the samples used for this pilot study were preselected to include a disproportionately higher number of nonresponders in both groups than would be anticipated in the general ALL patient population, serum ASNase activity of the PEG-ASNase group was almost 3-fold higher than the ASNase group. This higher level of ASNase activity in the PEG-ASNase group occurred despite a markedly lower administered dose (i.e., mean activity of 240 U/L at 1000 U/m^2 for a single dose of PEG-ASNase versus mean activity of 84 U/L after at least 7 doses of 5000–10,000 U/m^2 of unmodified ASNase). In addition, the mean sampling time after administration was longer for the PEG-ASNase group at 7.1 days versus 3.0 days for the ASNase group. Thus, in the majority of patients PEG-ASNase achieves higher and sustained ASNase activity than with conventional asparaginase. However, our data clearly show that anti-PEG is closely associated with the rapid clearance of PEG-ASNase for a subgroup of pediatric patients treated for ALL[32,33] without any clinical manifestation of an allergy or hypersensitivity. In addition, our findings provide further evidence regarding the importance of ASNase activity measurement to ensure ASN depletion for the recommended period.[34,35,42]

As anticipated, no correlation was observed between serum ASNase activity and the presence of anti-PEG for the patients receiving unmodified ASNase treatment. This lack of association is confirmed by the analysis presented in Table 1 that shows a poor overall accuracy (50%) for anti-PEG as a predictor of ASNase activity in ASNase-treated patients. Conversely, analysis of the patient data for those treated with PEG-ASNase clearly shows a strong correlation between the occurrence of anti-PEG and ASNase activity ($P = 3.6 \times 10^{-5}$, Fig. 3, and 92%/92% and 100%/100% specificity/positive predictive value for flow cytometric and serological detection of anti-PEG, Table 1).

It is important to note that all PEG-ASNase-treated patient sera that were anti-PEG-positive by serology and 12/13 by flow cytometry showed undetectable ASNase activity, indicating that anti-PEG is a clinically significant antibody. It could be argued that the agglutination of PEG-RBCs by sera may be due to the exposure of a neoantigen or crypt-antigen on the RBC surface consequent to PEG-modification, rather than by an anti-PEG. This alternative possibility does not seem tenable in view of the following: 1) anti-PEG binds to TentaGel-OH particles, which are composed primarily of PEG; 2) undetectable ASNase activity was found for all PEG-ASNase-treated patient sera that contained anti-PEG by serology. It is also notable that approximately one-third of the ASNase-group tested positive for anti-PEG, a frequency that is comparable to the occurrence we determined in healthy blood donors.[28–30] This observation suggests that the anti-PEG occurrence in the PEG-ASNase group was preexisting and most likely not induced by the PEG-ASNase treatment.

We have presented 2 techniques to test for the presence anti-PEG and have addressed potential artifacts associated with either technique in the previous paragraph. The sensitivity of the serological detection of anti-PEG via direct agglutination of PEG-coated RBCs may well be improved by using an amplification technique (antiglobulin test) which would also detect samples that only contain an IgG anti-PEG. We thus recommend further optimization studies before the routine use of the serological technique. With the current data, all serologically anti-PEG positive sera showed undetectable ASNase activity in the PEG-ASNase group, which demonstrates the potential value of this technique.

Low serum ASNase activity was observed for 3 PEG-ASNase samples that tested negative for anti-PEG (Fig. 2). Consistent with the low activity, 2 of these sera tested positive for anti-ASNase. The other

serum sample showed no evidence of either anti-PEG or anti-ASNase but had undetectable ASNase activity (<5 U/L). An anomalous result was also observed for 1 other sample that was weakly positive for anti-PEG IgM (mean fluorescence of 65) and was positive for anti-ASNase, but had ASNase activity (123 U/L) at the low end of the therapeutic range. Unfortunately, the amount of each serum sample available for analysis was limited and further studies were not possible for these latter 2 samples.

Although several review articles have stated that PEG is weakly immunogenic and nonantigenic,[9–13,17–19] nearly all directly or indirectly base these comments on articles by Richter and Akerblom.[22,23] In their clinical study,[22] Richter and Akerblom showed a 0.2% occurrence of anti-PEG in the healthy population (n = 453) and 3.3% in allergic patients (n = 92). The occurrence of anti-PEG in allergic patients that were treated with PEG-conjugated allergens increased to 50% after the initial treatment (n = 58), but dropped to 28.5% after 2 years of treatment (n = 28). The authors concluded that the presence of anti-PEG was not clinically important when using PEG-modified allergens in hyposensitization therapy.[22]

Induction of anti-PEG in animal models[23,25] has shown that anti-PEG rapidly clears PEG-conjugated RBCs, particles, and proteins.[24–27] Considering the possible significance of these animal study results, it is unclear why no further investigations regarding the clinical importance of anti-PEG appear to exist in the literature. In part, this may be because of the previous lack of simple and reliable assays for anti-PEG, or may result from assuming that a 0.2% occurrence of anti-PEG in the healthy population would have minimal impact compared with the rapid clearance or toxicity of unconjugated agents. It may also be partly because of difficulties associated with generating antibodies to PEG alone, with this difficulty likely caused by the rapid clearance of low molecular mass PEGs primarily via renal excretion.[44] However, conjugation of PEG to proteins and particles may augment the generation of anti-PEG because PEGylated material is present in the circulation for longer periods of time and because the route of excretion may be altered when compared with the unconjugated PEG.

Recently, Ganson et al.[31] reported the induction of anti-PEG, detectable between 3 and 7 days after injection, in 5 of 13 patients treated for chronic refractory gout with PEG-uricase. Rapid clearance of PEG-uricase was observed in the anti-PEG-positive patients, identified as a low titer IgG and IgM anti-PEG. An enzyme-linked immunosorbent assay (ELISA) test was used to detect anti-PEG, with positives identified as those with Ig binding to immobilized PEG-uricase and the absence of Ig binding to immobilized uricase. In our current study it is unclear whether anti-PEG was preexisting or induced after exposure to PEG-ASNase, as no baseline samples were available. The existence of anti-PEG in 6 of 16 (38%) ASNase patient sera who had no documented prior exposure to PEG-ASNase (Fig. 4) may indicate that anti-PEG was preexisting in the PEG-ASNase-treated patients, as we have previously determined a 25% occurrence of anti-PEG in the healthy blood donor population[28–30] Nonetheless, the observations by Ganson et al.[31] support our findings that anti-PEG is most likely responsible for rapid clearance of a PEG-conjugated drug and may well explain similar observations with other PEGylated agents (eg, PEG-interferon[45]).

It is interesting to briefly consider the background that led to the present study. Our initial finding of an anti-PEG stemmed, accidentally, from our earlier work to develop ‘universal’ blood by PEG-coating RBCs as a way to mask blood group antigens.[30] Preliminary studies undertaken in the Garratty laboratories showed that sera from 22 of 100 healthy blood donors directly agglutinated PEG-RBCs[28] and that the agglutination appeared to be because of an anti-PEG, predominantly IgM. We subsequently eliminated alternative possibilities for agglutination (eg, nonspecific binding, neoantigens or crypt-antigens) and confirmed the presence of an antibody against PEG.[30] Our study of 250 healthy blood donor sera showed a markedly higher occurrence of anti-PEG (ie, 25%)[29,30] than was reported 2 decades ago.[22] This increase is most likely due to greater exposure to PEG and PEG-containing compounds in cosmetics, pharmaceuticals, and processed food products. Both IgG and IgM were identified, with the IgG subclass predominantly IgG-2, although IgG-1 and IgG-3 were also observed.[29,30] Studies of agglutination inhibition of PEG-RBCs by preincubating anti-PEG-positive plasmas with a range of oxyethylene oligomers and PEG indicated that the epitope for anti-PEG was 4–5 repeat ethoxy units (-C-O-C-) irrespective of the end group moiety[46]; this finding is in close agreement with the results of Richter and Akerblom.[23] Thus, because we had previously shown the in vivo significance of anti-PEG (ie, rapid clearance of PEG-RBCs in immunized rabbits[25]), we naturally began to question whether anti-PEG could adversely affect PEG-conjugated therapeutic agents.

Lastly, it is of interest to consider effective therapeutic approaches for patients with anti-PEG. One

possibility would be the infusion of a PEG-containing compound to block or suppress anti-PEG before administration of a PEG-conjugated drug. To avoid immune complex formation, the selection of a short oxyethylene oligomer comparable in size to the anti-PEG epitope (ie, 4–5 repeat ethoxy units) conjugated to a core group may be of value. In addition, larger clinical studies are clearly warranted in order to expand on the relatively small number of patient sera tested in the present study, and thus to more clearly define the role of anti-PEG in the clearance of PEG-conjugated drugs.

Conclusions

Our finding that the presence of anti-PEG is very closely associated with rapid clearance of PEG-ASNase may explain similar observations with other PEG-conjugated drugs (eg, PEG-interferon[45]). We would recommend further comprehensive studies to fully elucidate the effect of preexisting and the development of anti-PEG on PEG-ASNase and other PEG-conjugated agents. For the majority of patients, PEG-conjugation is beneficial for the treatment and management of various diseases where clearance or toxicity of the unmodified drug is a significant barrier. Although the advantages of PEG-conjugation are apparent, if our findings here are confirmed in a comprehensive study, we recommend that patients should be screened for preexisting anti-PEG and routinely monitored for the development of anti-PEG throughout the course of treatment with any PEG-containing agent. If an anti-PEG is found, then either the clearance rate of the PEG-conjugated drug should be carefully monitored and the doses adjusted to compensate, or, if available, substitution with an effective non-PEGylated alternative therapeutic agent should be considered.

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