

# Effect of Premedications in a Murine Model of Asparaginase Hypersensitivity<sup>§</sup>

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## ABSTRACT

A murine model was developed that recapitulates key features of clinical hypersensitivity to *Escherichia coli* asparaginase. Sensitized mice developed high levels of anti-asparaginase IgG antibodies and had immediate hypersensitivity reactions to asparaginase upon challenge. Sensitized mice had complete inhibition of plasma asparaginase activity ( $P = 4.2 \times 10^{-13}$ ) and elevated levels of mouse mast cell protease 1 ( $P = 6.1 \times 10^{-3}$ ) compared with nonsensitized mice. We investigated the influence of pretreatment with triprolidine, cimetidine, the platelet activating factor (PAF) receptor antagonist CV-6209 [2-(2-acetyl-6-methoxy-3,9-dioxo-4,8-dioxo-2,10-diazaoctacos-1-yl)-1-ethyl-pyridinium chloride], or dexamethasone on the severity of asparaginase-induced allergies. Combining triprolidine and CV-6209 was best for mitigating

asparaginase-induced hypersensitivity compared with nonpretreated, sensitized mice ( $P = 1.2 \times 10^{-5}$ ). However, pretreatment with oral dexamethasone was the only agent capable of mitigating the severity of the hypersensitivity ( $P = 0.03$ ) and partially restoring asparaginase activity ( $P = 8.3 \times 10^{-4}$ ). To rescue asparaginase activity in sensitized mice without requiring dexamethasone, a 5-fold greater dose of asparaginase was needed to restore enzyme activity to a similar concentration as in nonsensitized mice. Our results suggest a role of histamine and PAF in asparaginase-induced allergies and indicate that mast cell-derived proteases released during asparaginase allergy may be a useful marker of clinical hypersensitivity.

## Introduction

Asparaginase is one of the integral components of combination chemotherapy regimens used in the treatment of acute lymphoblastic leukemia (ALL) and lymphoma. The mechanism of action of asparaginase is not completely understood, but the active enzyme depletes asparagine and possibly glutamine systemically (Wu et al., 1978; Asselin et al., 1989; Chan et al., 2014). Common adverse events to asparaginase include allergic reactions, often accompanied by the development of anti-asparaginase IgG antibodies (Pieters et al., 2011). Serum anti-asparaginase IgG levels have been found to increase before the onset of allergic reactions (Liu et al., 2012), suggesting that high antibody titers are required to induce clinical hypersensitivity. Furthermore, lower serum asparaginase activity was found in patients with anti-asparaginase antibodies compared with patients without detectable antibodies, and the enzyme activities were inversely correlated with the antibody levels

(Liu et al., 2012). Anti-asparaginase IgG antibodies have been proposed to directly neutralize asparaginase activity (Albertsen et al., 2002; Pieters et al., 2011), and lower systemic exposure to asparaginase is associated with lower exposure to concomitantly administered dexamethasone and an increased risk of central nervous system relapse (Yang et al., 2008; Kawedia et al., 2012), although some studies showed no associations between anti-asparaginase IgG antibodies and ALL outcomes (Cheung et al., 1986; Larson et al., 1998; Asselin, 1999; Woo et al., 2000; Panosyan et al., 2004).

Classic allergies involve cell-associated antigen-specific IgE antibodies and require low doses of antigen, low titers of circulating antibody, and are mediated by the release of histamine (Finkelman et al., 2005). An alternative pathway for allergy, which appears to play a role in asparaginase-induced reactions (Liu et al., 2012), requires repeated exposure to the antigen, high antigen-specific IgG antibody levels, a large antigen dose, and the release of platelet activating factor (PAF) (Finkelman et al., 2005; Finkelman, 2007). Immunologic studies have shown that antihistamines or PAF receptor antagonist can block the symptoms of an allergic reaction depending on the mechanism of allergy induced by the antigen (Strait et al., 2002). Understanding the pathway of asparaginase allergy will inform strategies for

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**ABBREVIATIONS:** ALL, acute lymphoblastic leukemia; AUC, area under the curve; CV-6209, 2-(2-acetyl-6-methoxy-3,9-dioxo-4,8-dioxo-2,10-diazaoctacos-1-yl)-1-ethyl-pyridinium chloride; mMCP-1, mouse mast cell protease 1; PAF, platelet activating factor.

ameliorating the severity of hypersensitivity reactions and can help identify possible markers for detecting sensitized patients before receiving the offending drug.

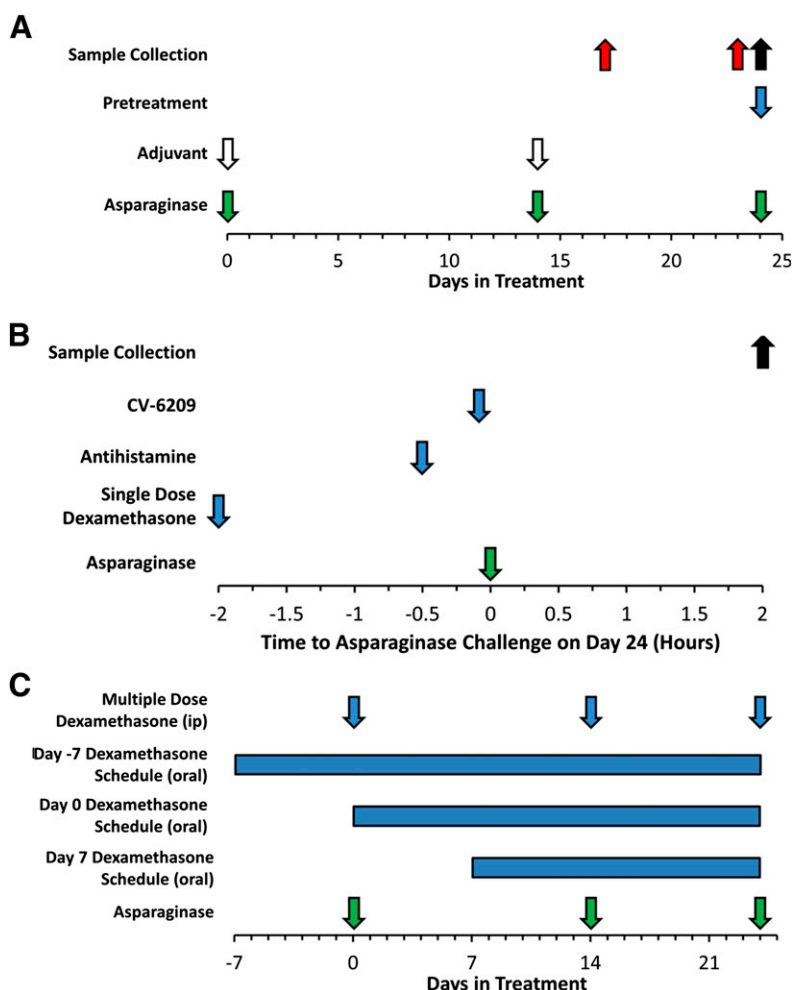
To investigate therapeutic strategies for mitigating allergies and maintaining plasma concentrations of asparaginase, we created a murine model of asparaginase allergy. The model recapitulates several features of clinical hypersensitivity reactions developed to *Escherichia coli* asparaginase. Our results indicate the involvement of both histamine and PAF in asparaginase-induced allergies and support the importance of monitoring asparaginase activity if pretreatment of patients with antihistamines or glucocorticoids is used to prevent allergy.

## Materials and Methods

**Asparaginase Sensitization Protocol.** Eight-week-old female BALB/c mice received 10  $\mu\text{g}$  i.p. doses of *E. coli* asparaginase (BioVendor Laboratory Medicine Inc., Candler, NC; >96.0% purity as determined by reverse phase high-performance liquid chromatography and SDS-PAGE) formulated with aluminum hydroxide adjuvant (Imject Alum; Thermo Scientific, Rockford, IL) on days 0 and 14 of treatment (Fig. 1A) to be sensitized to asparaginase. Control (nonsensitized) mice received intraperitoneal doses of adjuvant with vehicle alone (normal saline). Asparaginase allergies were induced in sensitized mice by challenging with a 100  $\mu\text{g}$  i.v. dose of *E. coli* asparaginase on day 24 of treatment. The onset of hypersensitivity was detected by monitoring decreases in rectal temperature using a digital thermometer (model BAT-12;

Physitemp Instruments, Clifton, NJ) for 2 hours after the asparaginase challenge. Prechallenge plasma samples for determining anti-asparaginase antibody levels were collected on day 23 of treatment by retro-orbital puncture (Fig. 1A), and postchallenge samples were collected by cardiac puncture at the end of the experiment for measuring antibody levels, asparaginase activity, and mouse mast cell protease 1 (mMCP-1) levels. The area under the temperature versus time curve was calculated using the trapezoidal rule. Lower area under the curve (AUC) values indicate more severe reaction (drop in rectal temperature), and differences in the severity of asparaginase-induced allergies between treatment groups was determined by comparing the AUC values of different groups. Mice were housed in an American Association of Laboratory Animal Care-accredited facility and treated using Institutional Animal Care and Use Committee-approved protocols in accordance with National Institutes of Health guidelines.

**Pretreatment Protocol.** Four different agents were investigated for their ability to mitigate asparaginase-induced allergic reactions: the antihistamines cimetidine (H2 receptor antagonist; Sigma-Aldrich, St. Louis, MO) and triprolidine (H1 receptor antagonist; Sigma-Aldrich), the PAF receptor antagonist CV-6209 [2-(2-acetyl-6-methoxy-3,9-dioxo-4,8-dioxo-2,10-diazoctacos-1-yl)-1-ethyl-pyridinium chloride; Santa Cruz Biotechnology, Santa Cruz, CA], and dexamethasone (American Pharmaceutical Partners, Inc., Schaumburg, IL). As described in Fig. 1B, antihistamine (cimetidine and triprolidine, 200  $\mu\text{g}$  i.p.) and CV-6209 (66  $\mu\text{g}$  i.v.) were given 30 and 5 minutes before the asparaginase challenge on day 24, respectively. Five different regimens of dexamethasone were investigated as described in Fig. 1, B and C. Single-dose dexamethasone was given 2 hours before the asparaginase challenge on day



**Fig. 1.** Schedule for sensitization to *E. coli* asparaginase and sample collection. (A) Mice received intraperitoneal injections of *E. coli* asparaginase (10  $\mu\text{g}$  or 2.25 IU per mouse, green arrows) formulated with aluminum hydroxide adjuvant (1 mg, white arrows) on day 0 and day 14 of the immunization schedule to sensitize mice to asparaginase. On day 24, mice were challenged with an intravenous dose of *E. coli* asparaginase (100  $\mu\text{g}$  or 22.5 IU per mouse, green arrow) to induce hypersensitivity reactions. Samples collected on day 23 (red arrow) were used to assess anti-asparaginase antibody titers before the asparaginase challenge on day 24. For a subset of mice, including those receiving oral dexamethasone, prechallenge samples were available only on day 17 of treatment (red arrow,  $n = 39$ ). Mice received pretreatment on day 24 (blue arrow) with CV-6209, antihistamine (triprolidine, cimetidine), or a single dose of dexamethasone (i.p.) in an attempt to mitigate the severity of the allergic reaction. (B) These pretreatment agents were administered 5 minutes, 30 minutes, or 2 hours before the asparaginase challenge, respectively. (C) Additional mitigation strategies included pretreatment with multiple doses of dexamethasone (i.p.) on days 0, 14, and 24 of treatment, and pretreatment with continuous oral dexamethasone starting 7 days before, same day, or 7 days after the initial asparaginase immunization dose. Samples collected 2 hours after the challenge on day 24 (black arrow) were used to measure asparaginase activity, antibody titers, and mMCP-1. Data in subsequent figures are color-matched accordingly to indicate the sample used for the laboratory value [black corresponding to day 24 samples (postchallenge dose of asparaginase) and red to day 23 or day 17 samples (prechallenge)].

24 (2 mg/kg i.p.), and multiple-dose dexamethasone was given 2 hours before each asparaginase dose on days 0, 14, and 24 (2 mg/kg i.p.). Pretreatment with oral continuous dexamethasone (4 mg/l in drinking water with a consumption rate of 5 ml/day yields an approximate dose of 0.8–1.0 mg/kg per day for the mice) starting 7 days before, same day (day 0), or 7 days after the initial asparaginase immunization dose was also evaluated for its influence on the severity of asparaginase-induced allergies. This regimen of dexamethasone achieves similar plasma dexamethasone concentrations as those achieved clinically (10–200 nM) in pediatric ALL patients given doses of 8 mg/m<sup>2</sup> per day (Yang et al., 2008, 2009; Kawedia et al., 2011).

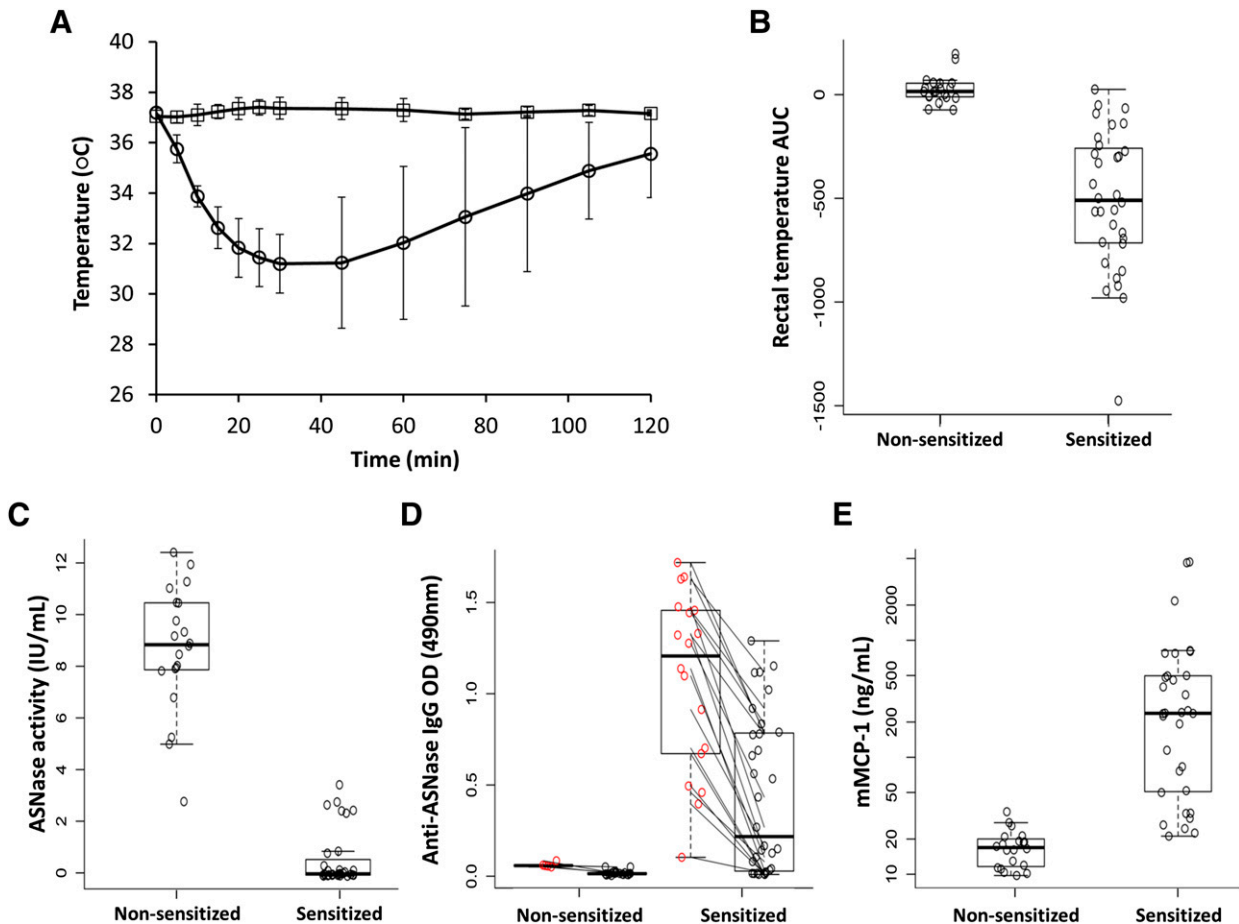
**Asparaginase Activity Determination.** The asparaginase activity in plasma samples was determined by monitoring the enzymatically coupled oxidation of NADH to NAD<sup>+</sup> in a 96-well format, as described previously (Fernandez et al., 2013). Briefly, 10  $\mu$ l of plasma from each sample was added to a 96-well microplate (Corning, Lowell, MA), and an enzyme reaction mixture containing asparagine,  $\alpha$ -ketoglutaric acid, glutamic oxalacetic transaminase,  $\beta$ -NADH, and malic dehydrogenase was added to each sample. A BioTek EL x 808 IU microplate reader (Winooski, VT) was used to determine the asparaginase activity. All asparaginase standards used for calibration purposes were prepared using mouse plasma, and samples with values over the standard curve were diluted with mouse plasma to get their enzyme activities within the linear range of the assay.

**Determining the Ex Vivo Asparaginase Activity Inhibition of Samples from Sensitized Mice.** The neutralization of asparaginase activity by postimmunization plasma was determined by adding 0.2 IU/ml of asparaginase to plasma samples collected after the asparaginase challenge (day 24). Plasma from sensitized mice, nonsensitized mice, or nonsensitized mice after addition of 5  $\mu$ g/ $\mu$ l of rabbit polyclonal anti-asparaginase IgG antibodies (Abcam, Cambridge, MA) were tested for residual asparaginase activity as described above.

**Detection of Anti-Asparaginase IgG Antibodies.** An enzyme-linked immunosorbent assay–based method was used to detect anti-*E. coli* asparaginase IgG antibodies, as described previously (Wang et al., 2000; Liu et al., 2012). Plasma samples from nonsensitized mice were used as negative controls, and rabbit polyclonal anti-asparaginase IgG antibodies were used as positive controls. Anti-mouse IgG (Sigma-Aldrich), anti-mouse IgE (Thermo Scientific), and anti-rabbit IgG secondary antibodies (Sigma-Aldrich) were used for detection of antibodies in samples or controls.

**Determining mMCP-1 Concentrations.** Concentrations of mMCP-1 were determined by enzyme-linked immunosorbent assay using the Ready-SET-Go commercial kit from eBioscience (San Diego, CA).

**Statistical Analysis.** Differences between rectal temperature AUC, asparaginase activity, anti-asparaginase IgG levels, and mMCP-1 concentrations were determined using a pairwise Wilcoxon rank sum test.



**Fig. 2.** Mice sensitized to *E. coli* asparaginase developed asparaginase-induced hypersensitivity reactions when challenged. (A) The rectal temperature of sensitized (○) and nonsensitized (□) mice was monitored for 2 hours after intravenous administration of *E. coli* asparaginase (100  $\mu$ g) at day 24 (as per Fig. 1A). (B) Compared with nonsensitized controls, sensitized mice with asparaginase-induced allergies experienced a drop in rectal temperature that was quantified by the AUC of the rectal temperature versus time curve ( $P = 3.3 \times 10^{-10}$ ). (C) Sensitized mice had low to no detectable asparaginase activity ( $P = 4.2 \times 10^{-13}$ ) and (D) developed high anti-asparaginase IgG antibody titers compared with nonsensitized mice (red open circles,  $P = 1.1 \times 10^{-7}$ ). Sensitized mice showed a drop in antibody levels when comparing the prechallenge and postchallenge samples at day 23 versus day 24 (red versus black open circles,  $P = 6.7 \times 10^{-5}$ ), suggesting the formation of asparaginase–anti-asparaginase IgG immune complexes. (E) Sensitized mice with asparaginase-induced allergies had elevated levels of mMCP-1 compared with nonsensitized mice in samples collected 2 hours after the challenge ( $P = 6.1 \times 10^{-3}$ ).

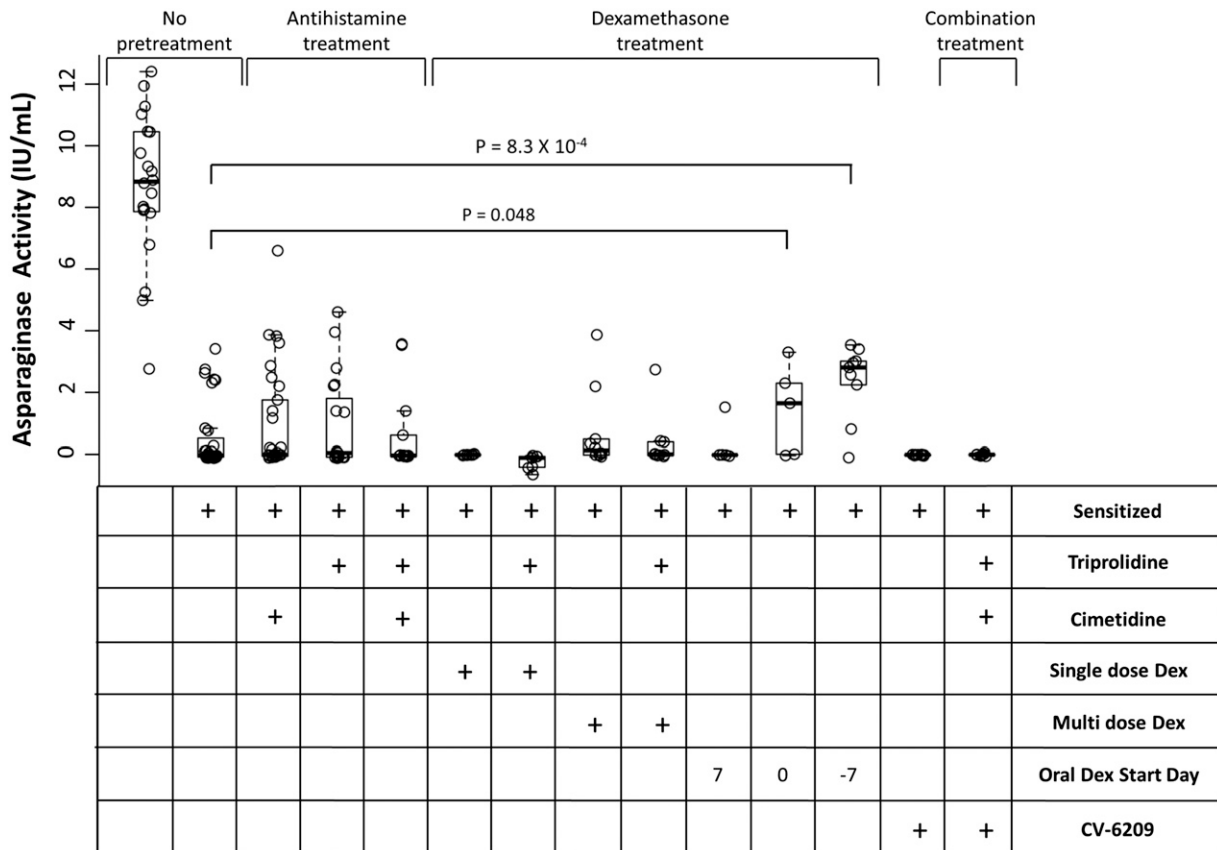


with no pretreatment (Fig. 3;  $P = 1.2 \times 10^{-5}$ ), antihistamine alone (Fig. 3;  $P = 1.6 \times 10^{-4}$ ), and single agent CV-6209 (Fig. 3;  $P = 3.2 \times 10^{-4}$ ). It was unclear whether triprolidine and/or cimetidine were responsible for reducing the severity of asparaginase-induced allergy; therefore, we tested each antihistamine as a single agent in sensitized mice. Cimetidine showed little effect compared with nonpretreated sensitized mice, and this group had more severe reactions compared with mice pretreated with a combination of cimetidine and triprolidine (Fig. 3;  $P = 1.1 \times 10^{-3}$ ). In contrast, mice pretreated with single agent triprolidine showed a decrease in the severity of allergies compared with nonpretreated, sensitized mice (Fig. 3;  $P = 0.02$ ), and their rectal temperature AUC was similar to that of mice pretreated with cimetidine and triprolidine in combination (Fig. 3;  $P > 0.05$ ). These results suggest that triprolidine but not cimetidine is required for mitigating asparaginase-induced allergies in our murine model.

PAF receptor antagonists, such as CV-6209, are not approved for clinical use in most countries. Therefore, we wanted to test whether dexamethasone could achieve similar effects as CV-6209 on asparaginase-induced allergies. Five dexamethasone pretreatment strategies were investigated: single-dose dexamethasone (Fig. 1B; 2 mg/kg i.p.) given 2 hours before the asparaginase challenge, multiple doses of dexamethasone (Fig. 1C; 2 mg/kg i.p.) given 2 hours before each immunization dose (Fig. 1C; days 0 and 14) and before the challenge (day 24),

and oral dexamethasone (Fig. 1C; 4 mg/l in drinking water) starting at 7 or 0 days before or 7 days after the initial asparaginase immunization dose. Pretreatment with single or multiple doses of dexamethasone was also evaluated with concomitant triprolidine. We found that mice pretreated with any of the dexamethasone regimens showed a decrease in the severity of the reaction compared with nonpretreated, sensitized mice (Fig. 3;  $P \leq 0.03$ ), except for mice receiving oral dexamethasone 7 days after the initial asparaginase immunization. We found no difference in rectal temperature AUC between single-dose dexamethasone pretreatment (with or without concomitant triprolidine), multiple-dose dexamethasone pretreatment (with or without concomitant triprolidine), and mice receiving oral dexamethasone (Fig. 3;  $P > 0.05$ ). Furthermore, all mice pretreated with any regimen containing dexamethasone had more severe reactions compared with mice pretreated with antihistamines and CV-6209 in combination (Fig. 3;  $P \leq 0.06$ ).

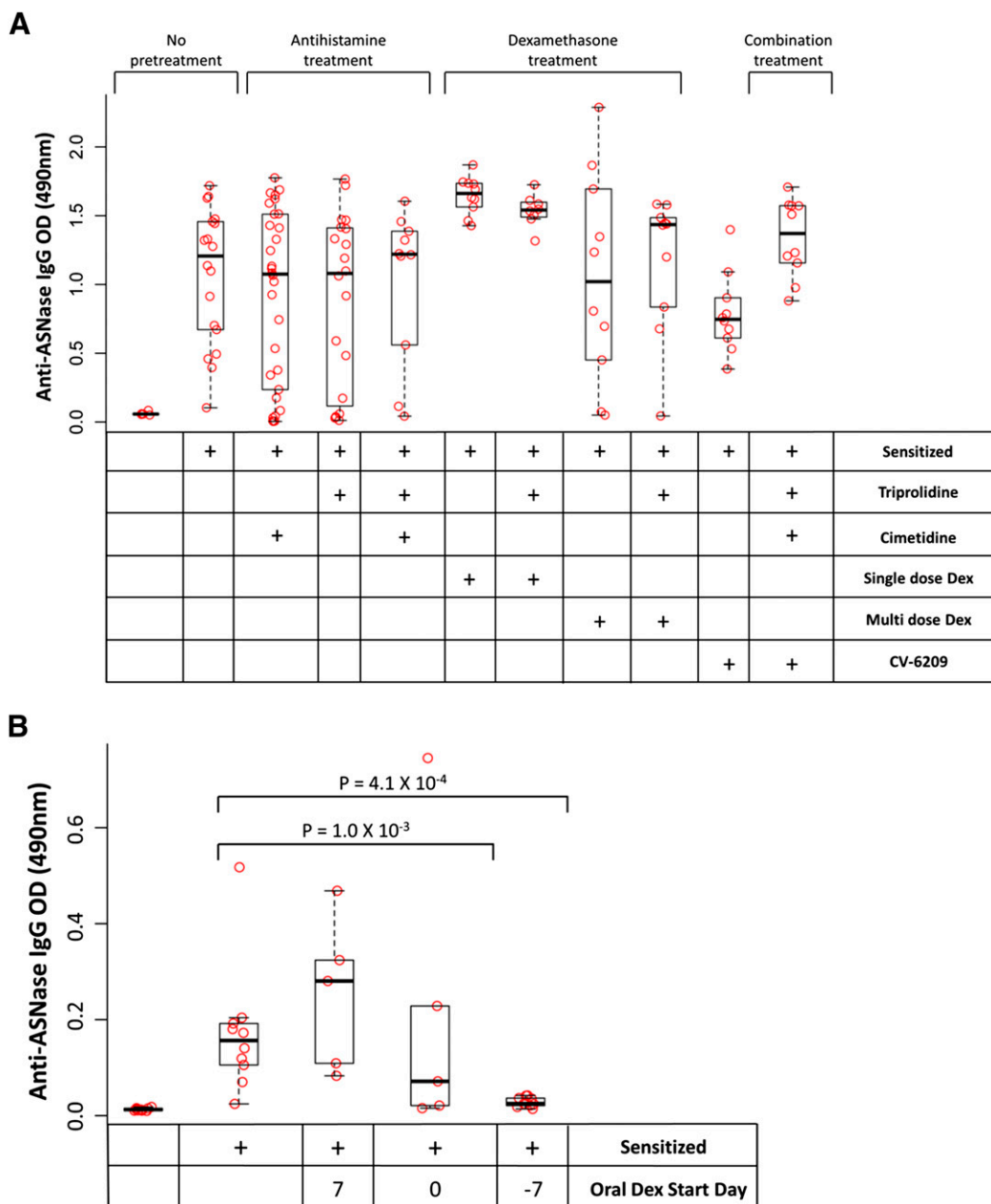
Asparaginase activity was determined in samples collected after the asparaginase-induced hypersensitivity to determine if pretreatment was able to restore activity (Fig. 4). Only oral dexamethasone given 0 or 7 days before the initial asparaginase was able to partially rescue activity compared with nonpretreated, sensitized mice (Fig. 4;  $P = 4.8 \times 10^{-2}$  and  $8.3 \times 10^{-4}$ , respectively). All other pretreatment strategies resulted in similar activity levels as nonpretreated, sensitized mice (Fig. 4;  $P > 0.05$ ). Nevertheless, mice receiving oral dexamethasone



**Fig. 4.** Oral dexamethasone given 7 days before asparaginase sensitization can partially restore asparaginase activity. Nonpretreated mice sensitized to asparaginase showed low to no detectable asparaginase activity compared with nonsensitized mice ( $P = 3.2 \times 10^{-14}$ ). Pretreatment with antihistamine, CV-6209, or intraperitoneal dexamethasone (Dex) was not able to rescue asparaginase activity relative to nonpretreated, sensitized mice ( $P > 0.05$ ). However, mice receiving continuous oral dexamethasone starting 0 or 7 days before asparaginase sensitization had partially restored asparaginase activity compared with nonpretreated, sensitized mice ( $P = 4.8 \times 10^{-2}$  and  $8.3 \times 10^{-4}$ , respectively).

0 or 7 days before the initial asparaginase had lower asparaginase activity than nonsensitized mice (Fig. 4;  $P = 3.8 \times 10^{-5}$  and  $1.9 \times 10^{-6}$ , respectively), suggesting that other methods of lowering anti-asparaginase IgG antibody levels may be required to completely rescue asparaginase activity. Similar pretreatment experiments with antihistamine, CV-6209, and dexamethasone were also tested in mice that were not sensitized to asparaginase to confirm that there was no direct influence of premedication on asparaginase activity (Supplemental Fig. 2). These results indicate that H1 and PAF receptor antagonists mitigate allergic reactions but do not influence the asparaginase activity.

The inability of pretreatment strategies to fully rescue asparaginase activity is likely due to high anti-asparaginase IgG levels that are unaffected by the pretreatment strategies we used. Mice immunized with asparaginase all developed high levels of anti-asparaginase IgG antibodies in prechallenge samples (day 23) compared with nonsensitized mice (Fig. 5A;  $P < 0.04$ ), including mice that received multiple doses of dexamethasone. However, mice pretreated with oral dexamethasone on the day of or 7 days before the initial asparaginase immunization showed diminished antibody levels compared with sensitized, nonpretreated mice (Fig. 5B;  $P = 1.0 \times 10^{-3}$  and  $4.1 \times 10^{-4}$ , respectively) in samples available on day 17 of treatment



**Fig. 5.** Dexamethasone effects the development of anti-asparaginase IgG antibodies. (A) On day 23 [before pretreatment with intraperitoneal single-dose dexamethasone (Dex), triprolidine, cimetidine, or CV-6209], all sensitized mice had elevated anti-asparaginase IgG levels compared with nonsensitized controls ( $P < 0.04$ ). Mice that received pretreatment with multiple doses of parenteral dexamethasone (with or without triprolidine) on days 0 and 14 before the day 23 blood collection had similar antibody levels as sensitized, nonpretreated mice ( $P > 0.05$ ). (B) Mice receiving continuous oral dexamethasone starting 0 or 7 days before the asparaginase sensitization had lower measured anti-asparaginase IgG levels compared with sensitized, nonpretreated mice ( $P = 1.0 \times 10^{-3}$  and  $4.1 \times 10^{-4}$ , respectively) in samples available on day 17 of treatment (before the asparaginase challenge).

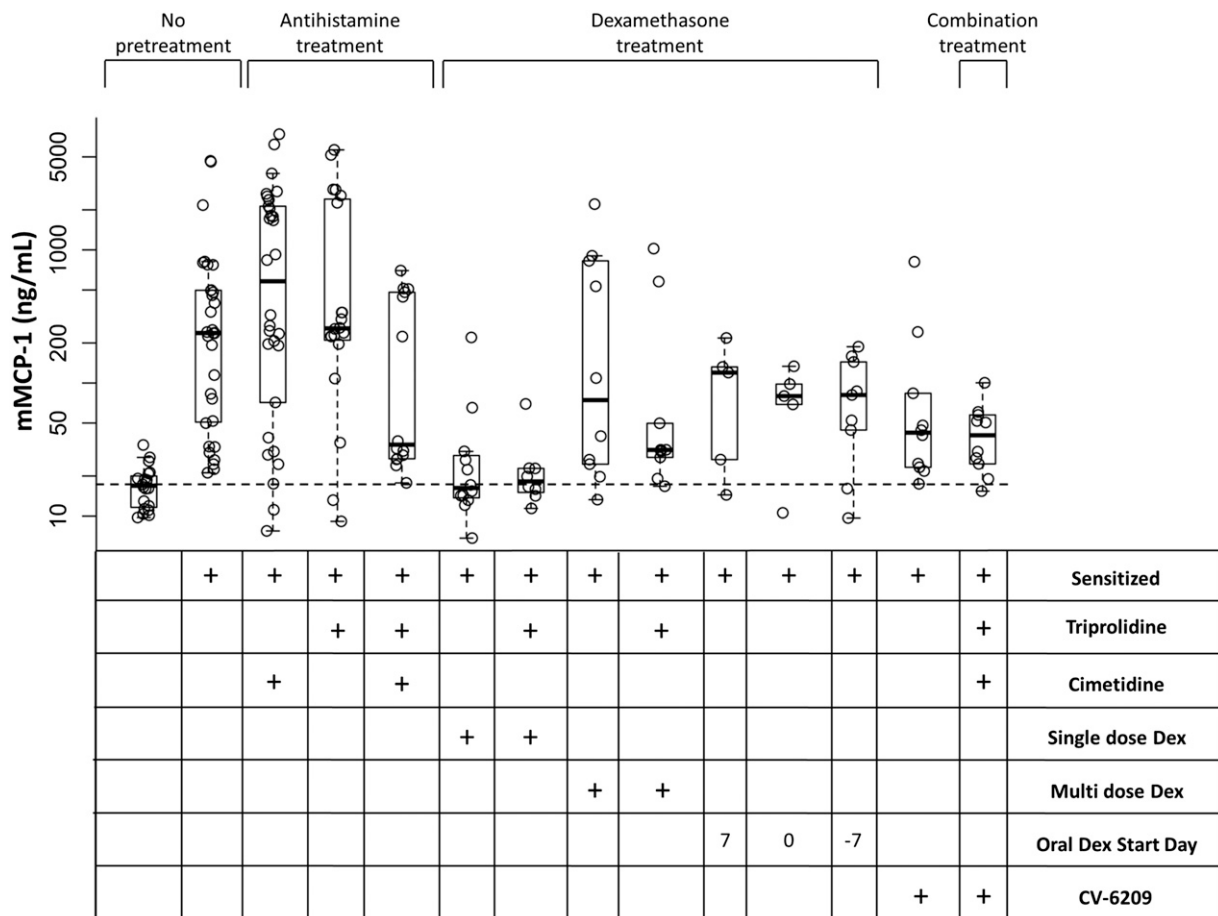
(Fig. 1A). These results indicate that oral dexamethasone was able to partially restore asparaginase activity due to its effect on anti-asparaginase IgG antibody levels.

We hypothesized that many of the pretreatment strategies were masking the severity of the hypersensitivity reactions rather than blocking the release of the chemical mediators of allergy. To confirm this, we measured concentrations of mMCP-1 in samples that were collected after the asparaginase-induced hypersensitivity. All sensitized mice showed elevated levels of mMCP-1 compared with nonsensitized mice (Fig. 6;  $P \leq 0.02$ ), except for mice receiving a single dose of dexamethasone (with or without concomitant triprolidine,  $P > 0.05$ ). Interestingly, this observation was not observed with any other dexamethasone pretreatment regimen. It is possible that the pretreatment with a single dose of dexamethasone mitigated the reactions by inhibiting the release of histamine during the allergic reaction, whereas other dexamethasone regimens masked the hypersensitivity through other mechanisms that likely affect antibody production. Similar data were previously reported, suggesting that dexamethasone can inhibit the release of histamine from mast cells and basophils (Schleimer et al., 1982, 1987; Berenstein et al., 1987).

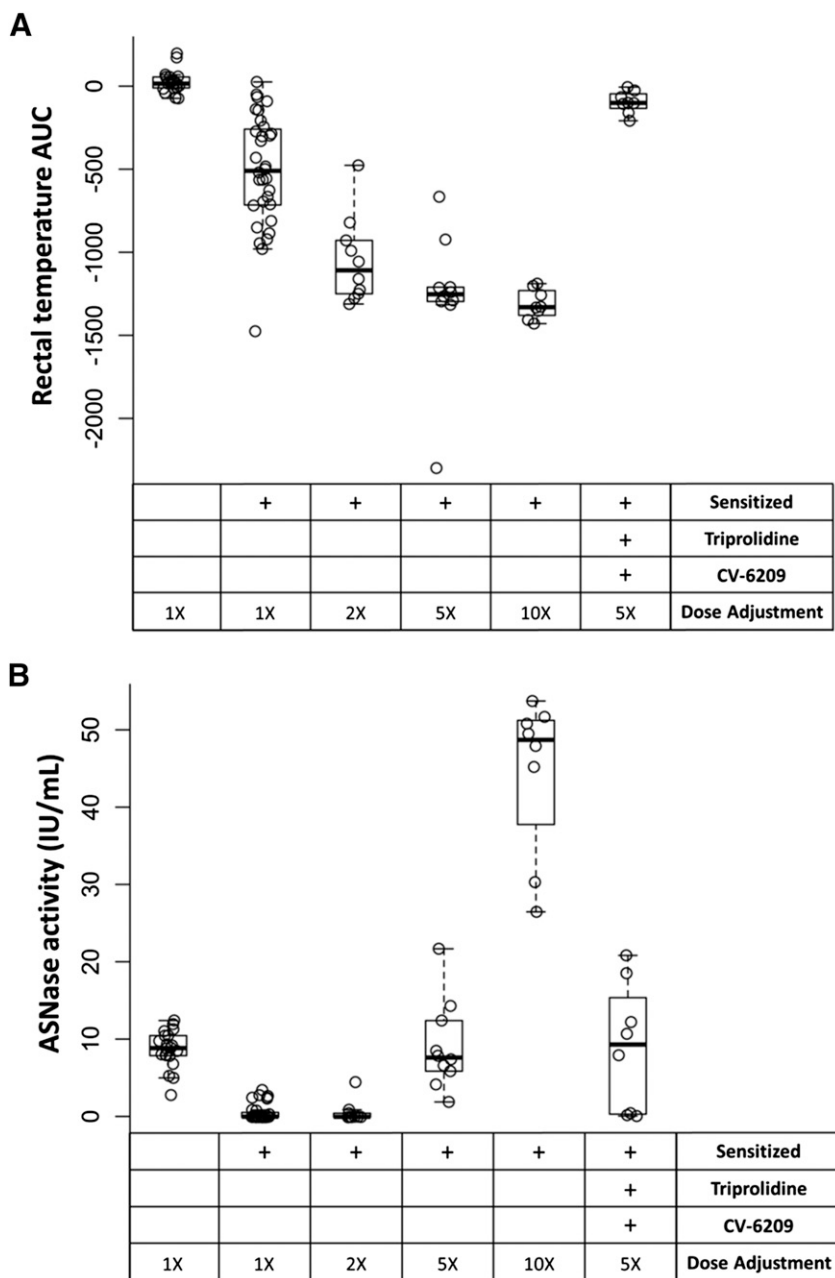
We next investigated if increasing the dose of asparaginase used during the challenge could overcome the high antibody titers and restore asparaginase activity without requiring dexamethasone. In the absence of pretreatment, the severity of

asparaginase-induced reactions increased in a dose-dependent manner, and all groups challenged with higher doses of asparaginase had more severe reactions compared with sensitized mice challenged with the standard 100- $\mu\text{g}$  dose (Fig. 7A;  $P \leq 2.1 \times 10^{-4}$ ). Furthermore, dose increases  $\geq 5$ -fold (5 $\times$  and 10 $\times$ ) were able to restore enzyme activity to concentrations similar to or higher than that of nonsensitized mice (Fig. 7B). Although increasing the dose of asparaginase used during the challenge from 1- to 10-fold showed a trend toward lower antibody titers in samples collected after the challenge (data not shown), the trend failed to reach statistical significance. mMCP-1 levels were elevated in a dose-dependent manner and correlated with the severity of the reactions (Fig. 8;  $R^2 = 0.577$ ,  $P = 3.0 \times 10^{-16}$ ).

Our pretreatment studies showed that triprolidine and CV-6209 were the best for mitigating the severity of asparaginase-induced allergies, and our dose adjustment experiments indicated that a 500- $\mu\text{g}$  dose of asparaginase per mouse was required to rescue asparaginase activity in sensitized mice. Therefore, we combined both strategies to determine if we could restore activity while simultaneously mitigating the severity of the allergy. We found that the pretreatment, even at the higher dose of asparaginase, was capable of blocking the allergic reaction compared with sensitized, nonpretreated mice (Fig. 7A;  $P = 9.8 \times 10^{-4}$ ), and the dose adjustment restored asparaginase activity to similar concentrations as nonsensitized mice (Fig. 7B;



**Fig. 6.** Elevated levels of mMCP-1 were detected in sensitized mice after asparaginase-induced allergies. Elevated levels of mMCP-1 were detected in all sensitized mice after asparaginase-induced allergies ( $P < 0.02$ ), except for mice pretreated with a single dose of intraperitoneal dexamethasone (Dex) or a single dose of intraperitoneal dexamethasone in combination with triprolidine.



**Fig. 7.** Asparaginase dose adjustments restored activity levels in sensitized mice. Sensitized mice were challenged with 2-, 5-, or 10-fold higher asparaginase doses. (A) The severity of the reaction (drop in rectal temperature) showed a dose-dependent relationship ( $P < 1.0 \times 10^{-3}$  compared with 1 $\times$ , sensitized), and mice pretreated with tripolidine and CV-6209 receiving a 5-fold higher dose were able to mitigate the reaction relative to nonpretreated mice ( $P = 9.8 \times 10^{-4}$ ). (B) Furthermore, dose adjustments  $\geq 5$ -fold were able to restore asparaginase activity in samples collected 2 hours after the challenge compared with nonsensitized mice ( $P > 0.05$ ).

$P > 0.05$ ). The anti-asparaginase antibody levels and mMCP-1 concentrations measured for pretreated and nonpretreated mice given a 5-fold increased dose were similar ( $P > 0.05$ , data not shown), suggesting that mice in both groups had similar potential for developing hypersensitivity reactions of comparable severities.

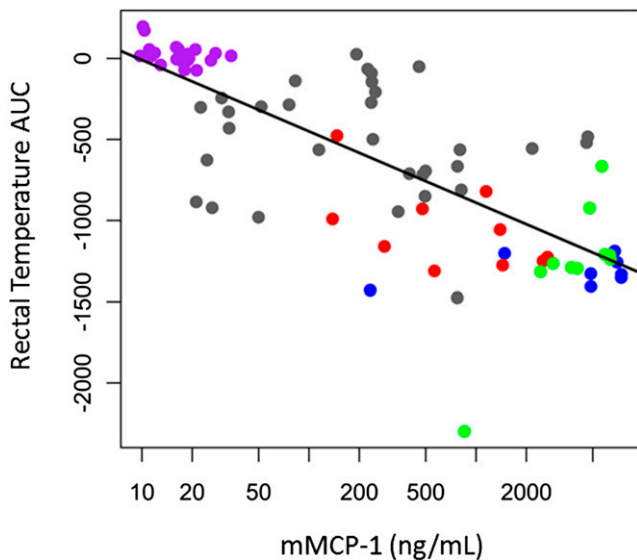
### Discussion

Clinical immune responses to asparaginase during the treatment of leukemia almost exclusively occur upon re-exposure to asparaginase after an initial exposure (Albertsen et al., 2002; Pieters et al., 2011; Fernandez et al., 2014a), implying that these patients were sensitized to asparaginase administered initially. Reactions in sensitized patients are usually characterized by an immediate allergic response after administration

of asparaginase, by the development of high levels of anti-asparaginase IgG antibodies, and by low serum asparaginase activity concentrations (Peterson et al., 1971; Spiegel et al., 1980; Muller et al., 2001; Panosyan et al., 2004; Pieters et al., 2011; Liu et al., 2012). The sensitization protocol we used in mice (Fig. 1, A and B) resulted in the development of an asparaginase-mediated immune response with a similar phenotype as clinical hypersensitivity to *E. coli* asparaginase.

Our results have several implications for improving use of asparaginase. Our data support that an H1 histamine receptor antagonist (triprolidine) used in combination with a PAF receptor antagonist (CV-6209) is best for mitigating asparaginase-induced reactions (Fig. 3). Although we did not measure IgE antibodies, these response data are consistent with a role for both anti-asparaginase IgG (through Fc $\gamma$ R-dependent release of PAF) and IgE antibodies (through Fc $\epsilon$ R1-dependent release of





**Fig. 8.** The severity of asparaginase-induced allergies was correlated with mMCP-1 concentrations. The lower the rectal temperature AUC, the more severe the allergic reaction, and the higher the mMCP-1 concentrations from samples collected after the challenge on day 24 for nonsensitized mice (purple circle) and for sensitized mice receiving 100  $\mu\text{g}$  (gray circle), 200  $\mu\text{g}$  (red circle), 500  $\mu\text{g}$  (green circle), or 1 mg (blue circle) of asparaginase per mouse during the challenge ( $R^2 = 0.577$ ,  $P = 3.0 \times 10^{-16}$ ).

histamine) being involved in eliciting asparaginase hypersensitivity reactions. However, pretreatment with these agents did not rescue plasma asparaginase activity (Fig. 4). These experiments clearly demonstrate that the symptoms and signs of asparaginase allergies can be completely masked by pretreatment and yet result in subtherapeutic drug concentrations, thus emphasizing the importance of monitoring asparaginase activity after pretreatment with antihistamines, glucocorticoids, or any other medication used for mitigating the severity of allergic reactions. In addition, the low asparaginase activity measured in sensitized mice is likely due to an accelerated clearance and antibody neutralization, because the maximum *ex vivo* inhibition we were able to induce using rabbit anti-asparaginase IgG antibodies (5  $\mu\text{g}/\mu\text{l}$ ) was only 50% (Supplemental Fig. 1). Similar neutralization results were previously reported (Peterson et al., 1971; Baechtel and Prager, 1973), suggesting that anti-asparaginase IgG antibodies cannot completely neutralize asparaginase activity and that other mechanisms are involved in the rapid *in vivo* disappearance of enzyme activity. This is further supported by the >50% loss of asparaginase activity (Fig. 4) seen in mice pretreated with dexamethasone 7 days before the initial asparaginase immunization dose, although they had negligible antibody levels (Fig. 5B) compared with other pretreatment groups. This is similar to the accelerated asparaginase clearance seen in pediatric patients with relapsed ALL receiving *E. coli* asparaginase and negative for anti-asparaginase IgG antibodies (Panetta et al., 2009).

We found that dexamethasone could be used to mitigate the reaction, although at the dosage schedules we studied it was not as effective in masking the allergic reaction as pretreatment with triprolidine and CV-6209 in combination (Fig. 3;  $P \leq 0.06$ ). However, pretreating mice with oral dexamethasone before exposure to asparaginase lowered anti-asparaginase IgG levels (Fig. 5B) and partially maintained asparaginase activity (Fig. 4).

These results are similar to recent clinical findings that showed a lower incidence of asparaginase hypersensitivity in patients that received dexamethasone 4 days before asparaginase administration during remission induction and support the use of glucocorticoids before beginning asparaginase (Pui, 2013; Vora et al., 2013).

Our dose adjustment studies indicate that strategies to lower anti-asparaginase IgG antibody titers (by overcoming their inhibitory effects by presenting a higher concentration of antigen) can restore enzyme activity. However, the effect of large asparaginase doses on treatment-related toxicities is unknown. The dose-adjustment experiments also show that the severity of asparaginase-induced reactions increases in a dose-dependent manner (Fig. 7A). Thus, when challenging patients after a suspected reaction, a lower dose of asparaginase might be expected to result in a less serious reaction but may not overcome the antibody levels and result in lower serum asparaginase activity levels.

Histamine and PAF are considered primary chemical mediators of allergic shock by acting on smooth muscle endothelial cells (Finkelman, 2007). However, histamine or PAF are unlikely to be useful biomarkers of asparaginase-induced allergies due to their short serum half-life. Histamine concentrations reach peak levels within 5 minutes of the onset of symptoms and have a serum half-life of a few minutes (Ferrer et al., 2010), whereas PAF has a serum half-life <15 minutes (Vadas et al., 2008). Several studies have shown that elevated levels of tryptase can be used as a biomarker of clinical hypersensitivity (Ordoqui et al., 1997; He et al., 2004; Payne and Kam, 2004; Fernandez et al., 2014b), and in accord with those studies, we found that mMCP-1 was elevated in sensitized mice from samples collected 2 hours after the asparaginase challenge (mMCP-1 is a mucosal mast cell granule-specific  $\beta$ -chymase that is released during basophil or mast cell degranulation in mice similar to tryptase in humans; Caughey, 2007). Furthermore, mMCP-1 concentrations were correlated with the severity of allergic reactions (Fig. 8), and previous studies showed that increases in plasma mMCP-1 levels may be induced by either IgG or IgE-mediated allergies (Strait et al., 2002). Unlike histamine and PAF, tryptase reaches peak concentrations ~30–60 minutes after allergy onset, has a serum half-life of 90 minutes, and exhibits low serum concentrations in normal, nonallergic individuals (Laroche et al., 1991). Although other studies have suggested that histamine levels may be a more useful immediate (e.g., 10 minutes) indicator of the onset of allergies compared with the release of mast cell proteases in mice (Nabe et al., 2013), a biomarker of allergy that is elevated and stable for several hours after an allergic reaction may be more useful as an eventual clinical biomarker of asparaginase-induced allergies. Taken together, our results suggest that tryptase may be a possible clinical biomarker for confirming episodes of hypersensitivity to asparaginase.

Aluminum hydroxide adjuvant was required for mice to develop immune responses to asparaginase, as mice immunized with asparaginase without adjuvant did not develop allergic reactions when challenged (data not shown). Selection of an adjuvant can play a large role in determining the characteristics of the immune response generated (e.g., antibody isotypes, subclass, affinity, and titer) (Hadjipetrou-Kourounakis and Moller, 1984; Kenney et al., 1989; Muller and Boos, 1998). Anti-asparaginase antibodies in ALL patients have been reported to be predominantly IgG1 and IgG4 (Cheung et al., 1986; Korholz

et al., 1987), which is consistent with a TH2 response (Visciano et al., 2012). Immunization with aluminum hydroxide generally elicits a TH2 response with the development of antigen-specific IgG1 and IgE antibodies (Kenney et al., 1989). In accordance with these reports, asparaginase-sensitized mice developed anti-asparaginase IgG1 antibodies, but no IgE antibodies were detected (data not shown). Therefore, clinical hypersensitivity reactions to asparaginase that are associated with the development of anti-asparaginase IgG antibodies and the asparaginase-induced allergic reaction in our murine model both appear to be TH2 driven. Nevertheless, there are several similarities and differences between the murine and human immune system that may influence the translation of our results to human allergies. Both species produce IgE antibodies that bind with high affinity to the FcεRI receptor, and cross-linking the receptor results in the release of chemical mediators of anaphylaxis, including histamine and PAF (Finkelman, 2007). Both species also produce IgG antibodies that induce the release of PAF by binding to the FcγRIII receptor, and both can mediate allergic reactions by the activation of complement through the production of anaphylatoxins. In contrast, the human and murine immune systems differ in the ability to activate complement through their respective IgG isotypes (Snapper and Finkelman, 1999), in the cells that express FcεR1 (Bieber et al., 1992; Maurer et al., 1994, 1996; Kinet, 1999), and in the quantity of granules within basophils, which influences the role of chemical mediators of allergy during hypersensitivity reactions (Lee and McGarry, 2007; Tsujimura et al., 2008). It is also possible that other chemical mediators of allergies in addition to PAF and histamine, such as cysteinyl leukotrienes and prostaglandin D<sub>2</sub>, play a role during human allergic reactions to asparaginase (Ono et al., 2009).

In conclusion, we created the first murine model of immediate hypersensitivity reaction to asparaginase. Several previous studies have sensitized mice to asparaginase and demonstrated the development of anti-asparaginase antibodies (Roberts et al., 1966; Baechtel and Prager, 1973; Goldberg et al., 1973; Uren and Ragin, 1979; Kamisaki et al., 1981; Yagura et al., 1981; Fernandes and Gregoriadis, 2001; Cantor et al., 2011) and demonstrated delayed hypersensitivity reactions to asparaginase (Kawamura et al., 1985; Gaspar et al., 1996). Our model is the first to show the development of acute symptomatic allergies in sensitized mice and to identify methods of reducing the severity of the symptoms and restoring asparaginase activity. We demonstrate that pretreatment with H1 receptor antagonists, PAF receptor antagonist, or dexamethasone can all partially mitigate the severity of asparaginase-induced allergic reactions to a similar extent; however, only dexamethasone was able to partially maintain plasma asparaginase activity. This may be particularly important for selecting a pretreatment agent in patients that are at a high risk of developing glucocorticoid-induced osteonecrosis (Vora, 2011). Our results also support the need for a clinically available PAF receptor antagonist for mitigating immune responses (Roberts et al., 1988; Hozawa et al., 1995; Kingsnorth et al., 1995) and emphasize that it may be important to monitor asparaginase activity if pretreatment medications are used. Furthermore, our results indicate that strategies that can lower anti-asparaginase antibodies might be useful to restore enzyme activity in a similar manner as our dose escalation approach.

#### Authorship Contributions

*Participated in research design:* Fernandez, Smith, Finkelman, Relling.

*Conducted experiments:* Fernandez.

*Performed data analysis:* Fernandez, Smith.

*Wrote or contributed to the writing of the manuscript:* Fernandez, Smith, Karol, Ramsey, Liu, Pui, Jeha, Evans, Finkelman, Relling.

#### References

- Albertsen BK, Schröder H, Jakobsen P, Avramis VI, Müller HJ, Schmiegelow K, and Carlsen NT (2002) Antibody formation during intravenous and intramuscular therapy with *Erwinia* asparaginase. *Med Pediatr Oncol* **38**:310–316.
- Asselin BL (1999) The three asparaginases. Comparative pharmacology and optimal use in childhood leukemia. *Adv Exp Med Biol* **457**:621–629.
- Asselin BL, Ryan D, Frantz CN, Bernal SD, Leavitt P, Sallan SE, and Cohen HJ (1989) In vitro and in vivo killing of acute lymphoblastic leukemia cells by L-asparaginase. *Cancer Res* **49**:4363–4368.
- Baechtel S and Prager MD (1973) Basis for loss of therapeutic effectiveness of L-asparaginase in sensitized mice. *Cancer Res* **33**:1966–1969.
- Berenstein EH, Garcia-Gil M, and Siraganian RP (1987) Dexamethasone inhibits receptor-activated phosphoinositide breakdown in rat basophilic leukemia (RBL-2H3) cells. *J Immunol* **138**:1914–1918.
- Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, Hanau D, and de la Salle C (1992) Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI). *J Exp Med* **175**:1285–1290.
- Cantor JR, Yoo TH, Dixit A, Iverson BL, Forsthuber TG, and Georgiou G (2011) Therapeutic enzyme deimmunization by combinatorial T-cell epitope removal using neutral drift. *Proc Natl Acad Sci USA* **108**:1272–1277.
- Caughey GH (2007) Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* **217**:141–154.
- Chan WK, Lorenzi PL, Anishkin A, Purwaha P, Rogers DM, Sukharev S, Rempe SB, and Weinstein JN (2014) The glutaminase activity of L-asparaginase is not required for anticancer activity against ASNS-negative cells. *Blood* **123**:3596–3606.
- Cheung NK, Chau IY, and Coccia PF (1986) Antibody response to *Escherichia coli* L-asparaginase. Prognostic significance and clinical utility of antibody measurement. *Am J Pediatr Hematol Oncol* **8**:99–104.
- Fernandes AI and Gregoriadis G (2001) The effect of polysialylation on the immunogenicity and antigenicity of asparaginase: implication in its pharmacokinetics. *Int J Pharm* **217**:215–224.
- Fernandez CA, Cai X, Elozory A, Liu C, Panetta JC, Jeha S, Molinelli AR, and Relling MV (2013) High-throughput asparaginase activity assay in serum of children with leukemia. *Int J Clin Exp Med* **6**:478–487.
- Fernandez CA, Smith C, Yang W, Daté M, Bashford D, Larsen E, Bowman WP, Liu C, Ramsey LB, Chang T, et al. (2014a) HLA-DRB1\*07:01 is associated with a higher risk of asparaginase allergies. *Blood* **124**:1266–1276.
- Fernandez CA, Stewart E, Panetta JC, Wilkinson MR, Morrison AR, Finkelman FD, Sandlund JT, Pui CH, Jeha S, Relling MV, et al. (2014b) Successful challenges using native *E. coli* asparaginase after hypersensitivity reactions to PEGylated *E. coli* asparaginase. *Cancer Chemother Pharmacol* **73**:1307–1313.
- Ferrer M, Nunez-Cordoba JM, Luquin E, Grattan CE, De la Borbolla JM, Sanz ML, and Schwartz LB (2010) Serum total tryptase levels are increased in patients with active chronic urticaria. *Clin Exp Allergy* **40**:1760–1766.
- Finkelman FD (2007) Anaphylaxis: lessons from mouse models. *J Allergy Clin Immunol* **120**:506–515; quiz 516–507.
- Finkelman FD, Rothenberg ME, Brandt EB, Morris SC, and Strait RT (2005) Molecular mechanisms of anaphylaxis: lessons from studies with murine models. *J Allergy Clin Immunol* **115**:449–457; quiz 458.
- Gaspar MM, Perez-Soler R, and Cruz ME (1996) Biological characterization of L-asparaginase liposomal formulations. *Cancer Chemother Pharmacol* **38**:373–377.
- Goldberg AL, Cooney DA, Glynn JP, Homan ER, Gaston MR, and Milman HA (1973) The effects of immunization to L-asparaginase on antitumor and enzymatic activity. *Cancer Res* **33**:256–261.
- Hadjipetrou-Kourounakis L and Möller E (1984) Adjuvants influence the immunoglobulin subclass distribution of immune responses in vivo. *Scand J Immunol* **19**:219–225.
- He SH, Xie H, and He YS (2004) Induction of tryptase and histamine release from human colon mast cells by IgE dependent or independent mechanisms. *World J Gastroenterol* **10**:319–322.
- Hozawa S, Haruta Y, Ishioka S, and Yamakido M (1995) Effects of a PAF antagonist, Y-24180, on bronchial hyperresponsiveness in patients with asthma. *Am J Respir Crit Care Med* **152**:1198–1202.
- Kamisaki Y, Wada H, Yagura T, Matsushima A, and Inada Y (1981) Reduction in immunogenicity and clearance rate of *Escherichia coli* L-asparaginase by modification with monomethoxypolyethylene glycol. *J Pharmacol Exp Ther* **216**:410–414.
- Kawamura K, Igarashi T, Fujii T, Kamisaki Y, Wada H, and Kishimoto S (1985) Immune responses to polyethylene glycol modified L-asparaginase in mice. *Int Arch Allergy Appl Immunol* **76**:324–330.
- Kawedia JD, Kaste SC, Pei D, Panetta JC, Cai X, Cheng C, Neale G, Howard SC, Evans WE, Pui CH, et al. (2011) Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. *Blood* **117**:2340–2347; quiz 2556.
- Kawedia JD, Liu C, Pei D, Cheng C, Fernandez CA, Howard SC, Campana D, Panetta JC, Bowman WP, Evans WE, et al. (2012) Dexamethasone exposure and asparaginase antibodies affect relapse risk in acute lymphoblastic leukemia. *Blood* **119**:1658–1664.
- Kenney JS, Hughes BW, Masada MP, and Allison AC (1989) Influence of adjuvants on the quantity, affinity, isotype and epitope specificity of murine antibodies. *J Immunol Methods* **121**:157–166.

- Kinet JP (1999) The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. *Annu Rev Immunol* **17**:931–972.
- Kingsnorth AN, Galloway SW, and Formela LJ (1995) Randomized, double-blind phase II trial of Lexipafant, a platelet-activating factor antagonist, in human acute pancreatitis. *Br J Surg* **82**:1414–1420.
- Korholz D, Urbanek R, Nurnberger W, Jobke A, Gobel U, and Wahn V (1987) [Formation of specific IgG antibodies in l-asparaginase treatment. Distribution of IgG subclasses]. *Monatsschrift Kinderheilkunde. Organ der Deutschen Gesellschaft für Kinderheilkunde* **135**:325–328.
- Laroche D, Vergnaud MC, Sillard B, Soufarapis H, and Bricard H (1991) Biochemical markers of anaphylactoid reactions to drugs. Comparison of plasma histamine and tryptase. *Anesthesiology* **75**:945–949.
- Larson RA, Fretzin MH, Dodge RK, and Schiffer CA (1998) Hypersensitivity reactions to L-asparaginase do not impact on the remission duration of adults with acute lymphoblastic leukemia. *Leukemia* **12**:660–665.
- Lee JJ and McGarry MP (2007) When is a mouse basophil not a basophil? *Blood* **109**:859–861.
- Liu C, Kawedia JD, Cheng C, Pei D, Fernandez CA, Cai X, Crews KR, Kaste SC, Panetta JC, Bowman WP, et al. (2012) Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. *Leukemia* **26**:2303–2309.
- Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin MH, Kilgus O, Kinet JP, and Stingl G (1994) Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. *J Exp Med* **179**:745–750.
- Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer GF, Wichlas S, Jouvin MH, Schmitt-Egenolf M, Kraft D, Kinet JP, et al. (1996) Peripheral blood dendritic cells express Fc epsilon RI as a complex composed of Fc epsilon RI alpha- and Fc epsilon RI gamma-chains and can use this receptor for IgE-mediated allergen presentation. *J Immunol* **157**:607–616.
- Müller HJ, Beier R, Löning L, Blütters-Sawatzki R, Dörffel W, Maass E, Müller-Wehrich S, Scheel-Walter HG, Scherer F, Stahnke K, et al. (2001) Pharmacokinetics of native *Escherichia coli* asparaginase (Asparaginase medac) and hypersensitivity reactions in ALL-BFM 95 reinduction treatment. *Br J Haematol* **114**:794–799.
- Müller HJ and Boos J (1998) Use of L-asparaginase in childhood ALL. *Crit Rev Oncol Hematol* **28**:97–113.
- Nabe T, Matsuya K, Akamizu K, Fujita M, Nakagawa T, Shioe M, Kida H, Takiguchi A, Wakamori H, Fujii M, et al. (2013) Roles of basophils and mast cells infiltrating the lung by multiple antigen challenges in asthmatic responses of mice. *Br J Pharmacol* **169**:462–476.
- Ono E, Taniguchi M, Mita H, Fukutomi Y, Higashi N, Miyazaki E, Kumamoto T, and Akiyama K (2009) Increased production of cysteinyl leukotrienes and prostaglandin D2 during human anaphylaxis. *Clin Exp Allergy* **39**:72–80.
- Ordoqui E, Zubeldia JM, Aranzabal A, Rubio M, Herrero T, Tornero P, Rodríguez VM, Prieto A, and Baeza ML (1997) Serum tryptase levels in adverse drug reactions. *Allergy* **52**:1102–1105.
- Panetta JC, Gajjar A, Hijiya N, Hak LJ, Cheng C, Liu W, Pui CH, and Relling MV (2009) Comparison of native *E. coli* and PEG asparaginase pharmacokinetics and pharmacodynamics in pediatric acute lymphoblastic leukemia. *Clin Pharmacol Ther* **86**:651–658.
- Panosyan EH, Seibel NL, Martin-Aragon S, Gaynon PS, Avramis IA, Sather H, Franklin J, Nachman J, Ettinger LJ, La M, et al.; Children's Cancer Group Study CCG-1961 (2004) Asparaginase antibody and asparaginase activity in children with higher-risk acute lymphoblastic leukemia: Children's Cancer Group Study CCG-1961. *J Pediatr Hematol Oncol* **26**:217–226.
- Payne V and Kam PC (2004) Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia* **59**:695–703.
- Peterson RG, Handschumacher RE, and Mitchell MS (1971) Immunological responses to L-asparaginase. *J Clin Invest* **50**:1080–1090.
- Pieters H, Hunger SP, Boos J, Rizzari C, Silverman L, Baruchel A, Goekbuget N, Schrappe M, and Pui CH (2011) L-asparaginase treatment in acute lymphoblastic leukemia: a focus on *Erwinia asparaginase*. *Cancer* **117**:238–249.
- Pui CH (2013) Reducing delayed intensification therapy in childhood ALL. *Lancet Oncol* **14**:178–179.
- Roberts J, Prager MD, and Bachynsky N (1966) The antitumor activity of *Escherichia coli* L-asparaginase. *Cancer Res* **26**:2213–2217.
- Roberts NM, Page CP, Chung KF, and Barnes PJ (1988) Effect of a PAF antagonist, BN52063, on antigen-induced, acute, and late-onset cutaneous responses in atopic subjects. *J Allergy Clin Immunol* **82**:236–241.
- Schleimer RP, MacGlashan DW, Jr, Gillespie E, and Lichtenstein LM (1982) Inhibition of basophil histamine release by anti-inflammatory steroids. II. Studies on the mechanism of action. *J Immunol* **129**:1632–1636.
- Schleimer RP, Udem BJ, Meeker S, Bollinger ME, Adkinson NF, Jr, Lichtenstein LM, and Adams GK, 3rd (1987) Dexamethasone inhibits the antigen-induced contractile activity and release of inflammatory mediators in isolated guinea pig lung tissue. *Am Rev Respir Dis* **135**:562–566.
- Snapper C and Finkelman F (1999) Immunoglobulin Class Switching, in *Fundamental Immunology* (Paul W ed) Lippincott-Raven, Philadelphia.
- Spiegel RJ, Echelberger CK, and Poplack DG (1980) Delayed allergic reactions following intramuscular L-asparaginase. *Med Pediatr Oncol* **8**:123–125.
- Strait RT, Morris SC, Yang M, Qu XW, and Finkelman FD (2002) Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* **109**:658–668.
- Tsujimura Y, Obata K, Mukai K, Shindou H, Yoshida M, Nishikado H, Kawano Y, Minegishi Y, Shimizu T, and Karasuyama H (2008) Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunology* **28**:581–589.
- Uren JR and Ragin RC (1979) Improvement in the therapeutic, immunological, and clearance properties of *Escherichia coli* and *Erwinia carotovora* L-asparaginases by attachment of poly-DL-alanyl peptides. *Cancer Res* **39**:1927–1933.
- Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, Simons FE, Simons KJ, Cass D, and Yeung J (2008) Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* **358**:28–35.
- Visciano ML, Tagliamonte M, Tornesello ML, Buonaguro FM, and Buonaguro L (2012) Effects of adjuvants on IgG subclasses elicited by virus-like particles. *J Transl Med* **10**:4.
- Vora A (2011) Management of osteonecrosis in children and young adults with acute lymphoblastic leukaemia. *Br J Haematol* **155**:549–560.
- Vora A, Goulden N, Wade R, Mitchell C, Hancock J, Hough R, Rowntree C, and Richards S (2013) Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. *Lancet Oncol* **14**:199–209.
- Wang B, Hak LJ, Relling MV, Pui CH, Woo MH, and Storm MC (2000) ELISA to evaluate plasma anti-asparaginase IgG concentrations in patients with acute lymphoblastic leukemia. *J Immunol Methods* **239**:75–83.
- Woo MH, Hak LJ, Storm MC, Sandlund JT, Ribeiro RC, Rivera GK, Rubnitz JE, Harrison PL, Wang B, Evans WE, et al. (2000) Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. *J Clin Oncol* **18**:1525–1532.
- Wu MC, Arimura GK, and Yunis AA (1978) Mechanism of sensitivity of cultured pancreatic carcinoma to asparaginase. *Int J Cancer* **22**:728–733.
- Yagura T, Kamisaki Y, Wada H, and Yamamura Y (1981) Immunological studies on modified enzymes. I. soluble L-asparaginase/mouse albumin copolymer with enzyme activity and substantial loss of immunogenicity. *Int Arch Allergy Appl Immunol* **64**:11–18.
- Yang L, Boyd K, Kaste SC, Kamdem Kamdem L, Rahija RJ, and Relling MV (2009) A mouse model for glucocorticoid-induced osteonecrosis: effect of a steroid holiday. *J Orthop Res* **27**:169–175.
- Yang L, Panetta JC, Cai X, Yang W, Pei D, Cheng C, Kornegay N, Pui CH, and Relling MV (2008) Asparaginase may influence dexamethasone pharmacokinetics in acute lymphoblastic leukemia. *J Clin Oncol* **26**:1932–1939.

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