

Letters

TOTAL TRYPTASE LEVELS INDICATE RISK FOR SYSTEMIC REACTIONS TO RUSH IMMUNOTHERAPY AND MAST CELL ACTIVATION

Anaphylaxis is a serious systemic allergic reaction that is rapid in onset as defined in Sampson et al.¹ The current study examines individuals undergoing rush immunotherapy (RI) to aeroallergens to see whether those with systemic allergic reactions could be distinguished from nonreactors by measuring total and mature tryptase levels (see E-supplement).²

This study was reviewed and approved by the Franciscan Health System institutional review board. Fifteen consecutive patients chose to undergo RI to aeroallergens and provided signed informed consent. The extracts used (eTable 1), RI protocol (eTable 2), serum collection, and total and mature serum tryptase measurements^{3,4} are described in the E-supplement.

Five of the 15 subjects (30%) experienced mild systemic symptoms (reactors), including flushing, pruritus, urticaria, mild swelling, and nausea (eTable 3). No significant changes occurred in vital signs or pulmonary functions in reactors. Two of the 5 reactors (A, C) had a mild reaction on day 1, chose to undergo a second day of RI, and had a second reaction. The average pretotal tryptase level was significantly higher in the reactors (4.7 $\mu\text{g/L}$; range, 2.9–6.9 $\mu\text{g/L}$) than in the nonreactors (2.7 $\mu\text{g/L}$; range <1–5.2 $\mu\text{g/L}$) ($P = .02$) (Fig 1A). In addition, the average total tryptase level in the reactors postreaction (14.3 $\mu\text{g/L}$; range 9.0–23.0 $\mu\text{g/L}$) was significantly higher than the pre-RI value of this group ($P = .02$) as well as the pre-RI and post-RI levels of the nonreactors, 2.8 ng/mL (range <1–5.2) and 3.2 $\mu\text{g/L}$ (range <1–8.4 $\mu\text{g/L}$), respectively ($P < .001$) (Fig 1A). Six of the seven reactions among the 5 reactors involved elevations of total tryptase of greater than 2 times the baseline value (range 1.4–6.1-fold) (Fig 1B). Only 1 of the 7 reactions showed an increase in mature tryptase (patient A on day 2), but this did not correspond with a higher symptom severity, perhaps because of the pretreatment regimen. Only one of the nonreactors (patient O) had more than a 2-fold increase in total tryptase. Interestingly, the baseline total tryptase level of this subject was 1.6, lower than average, and urticaria onset was delayed, developing the day after the procedure. Although the current study did not identify a total tryptase level below which an individual would not be at risk for an allergic reaction, those with a baseline total tryptase level $>4 \mu\text{g/L}$ were more likely (OR = 16, 95% CI = 1.1–234) to experience a reaction during rush immunotherapy (Fig 1B).

To determine whether protryptases might be secreted by activated mast cells hours after they degranulate, skin mast cells in vitro were stimulated with anti-Fc ϵ RI monoclonal antibody. However, as

shown in Figure 1C, no significant increase in the secretion of total versus mature tryptase occurred over a 24-hour time course.

An increase of at least 2-fold in serum total tryptase levels among those with mild systemic reactions to rush immunotherapy (5 of 5 cases, 6 of 7 reactions) was a more sensitive biomarker than a serum level of mature tryptase of $\geq 1 \text{ ng/mL}$ (1 of 5 cases). The particular reaction for which the mature tryptase level was elevated yielded the highest total tryptase level. This is consistent with prior observations that total tryptase and mature tryptase levels increase coordinately during hypotensive anaphylaxis to insect stings and the ratio of total to mature tryptase approaches unity with the most severe cases.^{4,5} Another important point of the current study is that even though total tryptase levels increased at least 2-fold above baseline in reactors, peak levels remained in the normal range for 4 of the 7 reactions. This emphasizes the importance of having a baseline level for comparison. Such a baseline level could be obtained before the reaction, as in the current study, or at least 24 hours after all signs and symptoms of a reaction have ceased. Furthermore, the specificity of a doubling in the total tryptase level is supported by this occurring in only 1 of the 10 atopic nonreactors, although this particular subject did have a delayed urticarial reaction.

Why an increase in the total tryptase level is a more sensitive indicator of mild systemic reactions during rush immunotherapy than the mature tryptase level remains to be understood. In vitro, the vast majority of tryptase released from human skin mast cells and basophils within 30 minutes of stimulation is mature. In vivo, the time course after stimulation is often hours, raising the possibility that more protryptase is released at these later time points. However, this was not observed to occur with skin mast cells in vitro in the current study. Other possibilities may relate to differences in the solubility and diffusion of mature versus protryptases, because the mature tetramer binds heparin proteoglycan more tightly and would more likely form larger and more stable protease:proteoglycan complexes than monomeric protryptases.

In the current study, baseline serum total tryptase levels were significantly higher in those destined to have a systemic hypersensitivity reaction to rush immunotherapy compared with the nonreactors. Baseline tryptase levels are determined by the number of mast cells spontaneously secreting protryptases, the amount of protryptases secreted per cell, the efficiency by which released protryptase enters the circulation, and the rates of removal of circulating protryptases from the detectable pool. The primary determinant of this level is likely the total body burden of mast cells, but other factors also may come into play. Individuals with clonal mast cell disease associated with the D816V Kit mutation and a baseline serum level of total tryptase ≥ 11.4 as well as those with just an elevated total tryptase level are at increased risk for severe systemic anaphylaxis to insect stings,^{6,7} and to insect venom immunotherapy.⁸ Knowing which patients are at higher risk for systemic anaphylaxis could be used to help determine who is a good candidate for RI versus standard immunotherapy to aeroallergens, and who should be considered for more intense prophylactic treatment. The baseline total tryptase level appears to be one metric that merits further evaluation for this purpose.

Funding Sources: This work was funded in part by internal support at VCU and NIH AI20487 (LBS) and the Puget Sound Allergy Asthma and Immunology practice.

Disclosures: V.C.U. receives royalty payments from Phadia for sales of their tryptase assay, and these are shared with L.B.S., the inventor of that assay. The other authors have nothing to disclose.

© 2011 American College of Allergy, Asthma & Immunology.

Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2010.12.015

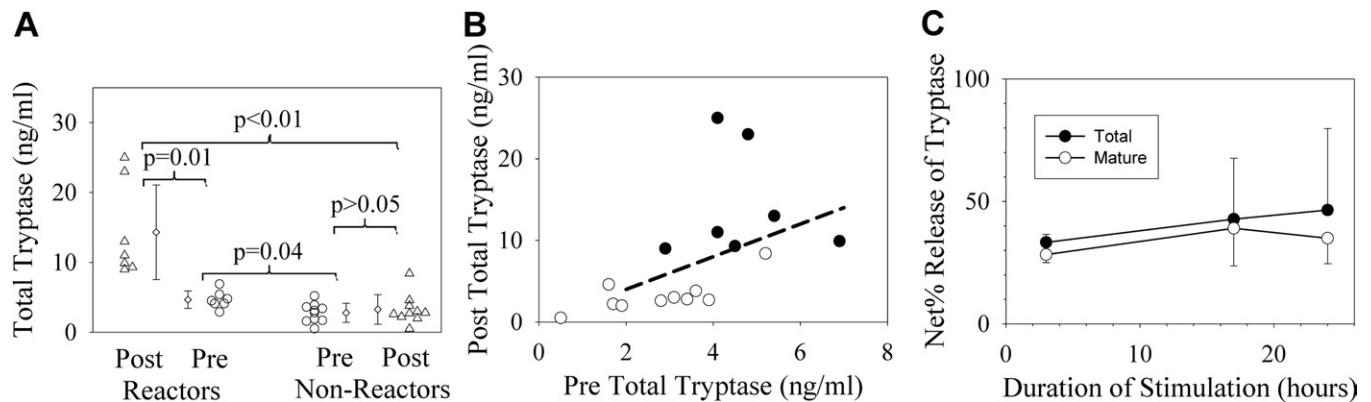


Figure 1. Total tryptase levels in vivo and in vitro. A, Total serum tryptase levels in reactors and nonreactors to RI, pre- and post-RI, or systemic reactions. A one-tailed unpaired *t* test with a Bonferroni correction was used. B, Post- versus pre-RI total tryptase levels in reactors (solid circles) and nonreactors (open circles). The dashed line shows a ratio of post to pre total tryptase of 2 for pre values ≥ 2 ng/mL. C, Tryptase release from skin mast cells over time. Skin-derived mast cells from three different individuals in culture with recombinant human stem cell factor (100 ng/mL; gift of Swedish Orphan Biovitrum [Stockholm, Sweden]) were stimulated with anti-Fc ϵ RI monoclonal antibody (22E7) and assessed for release of total and mature tryptase up to 24 hours later as described.³ Spontaneous release was $<5\%$ in each case.

RESULTS

Mature and total serum tryptase levels for each subject are shown in eTable 3.

ACKNOWLEDGMENTS

We thank Jiang Hu for her excellent technical assistance performing the serum tryptase measurements.

ARTHUR B. VEGH, MD*
 KIMBERLY C. GEORGE, MD†
 SAHAR LOTFI-EMRAN, BA†
 NICOLETTE E. BUTLER, BA*
 LAWRENCE B. SCHWARTZ, MD, PhD†

*Puget Sound Allergy Asthma and Immunology
 Tacoma, Washington
 †Department of Internal Medicine
 Virginia Commonwealth University
 Richmond, Virginia
 lbschwar@vcu.edu

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.anai.2010.12.015.

1. Sampson HA, Munoz-Furlong A, Campbell RL et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol.* 2006;391–397.
2. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am.* 2006;451–463.
3. Schwartz LB, Min HK, Ren S, et al. Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. *J Immunol.* 2003;5667–5673.
4. Schwartz LB, Bradford TR, Rouse C, et al. Development of a new, more sensitive immunoassay for human tryptase: Use in systemic anaphylaxis. *J Clin Immunol.* 1994;190–204.
5. Van der Linden P-WG, Hack CE, Poortman J, Vivié-Kipp YC, Struyvenberg A, Van der Zwan JK. Insect-sting challenge in 138 patients: Relation between clinical severity of anaphylaxis and mast cell activation. *J Allergy Clin Immunol.* 1992; 110–118.
6. Bonadonna P, Perbellini O, Passalacqua G, et al. Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. *J Allergy Clin Immunol.* 2009;680–686.
7. Rueff F, Przybilla B, Bilo MB, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase—a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol.* 2009; 1047–1054.
8. Rueff F, Przybilla B, Bilo MB, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol.* 2010;105–111.

E-SUPPLEMENT

Tryptase Background and Methods

The α and β tryptases are abundantly produced by human mast cells, and to a much lesser extent by basophils (<1%) and thereby serve as markers for mast cell involvement in various clinical situations. The human mast cell tryptases of interest are encoded by two genes located on chromosome 16: TPSB2, which is monomorphic for β -tryptase (ie, always β -tryptase) and TBSAB1, which is allelic for α and β tryptases (approximate 1:1 ratio of α and β tryptases). Consequently, tryptase genotypes (two tryptase genes on the paternal and two on the maternal chromosome 16) are $\alpha\beta:\alpha\beta$ in approximately 25% of persons; $\alpha\beta:\beta\beta$ in approximately 50%; and $\beta\beta:\beta\beta$ in approximately 25%.²

β -Protryptase is processed to a mature active tetramer, which is stored in secretory granules and released from activated mast cells by degranulation. Whether α -protryptase matures in mast cells is uncertain. Portions of α and β protryptases are constitutively released by unstimulated mast cells without being processed to maturity.³ Current immunoassays detect either total tryptase (α and β mature and pro tryptases) or mature tryptases.³ Healthy subjects have undetectable levels of mature tryptase (<1 ng/mL) and detectable levels of total tryptase (1–11.5 ng/mL) in serum or plasma at baseline. Elevated serum levels of mature tryptase reflect mast cell activation, for example, during insect sting–induced anaphylaxis, and correlate with clinical severity as reflected by a decline in mean arterial pressure, but remain <1 ng/mL in mild systemic reactions.⁵ Elevated total tryptase levels in baseline serum reflect expansion of the mast cell pool in mastocytosis² and also an increased risk for severe insect sting-initiated anaphylaxis.^{4,6,7} and systemic allergic reactions to venom immunotherapy.⁸ Elevated total tryptase levels in acute over baseline serum, like those of mature tryptase, also reflect anaphylactic hypotension; unlike mature tryptase levels, however, acute levels were 2-fold above

baseline in approximately 40% of subjects with mild normotensive systemic reactions to insect stings,⁴ even though both values were typically in the normal range.

METHODS

All study subjects were selected from Dr. Vegh's practice. These individuals presented with signs and symptoms of aeroallergy, had positive skin tests, and chose RI as their treatment option in consultation with Dr. Vegh. RI varied from 1 ($n = 3$) to 2 ($n = 12$) days. Patients were pretreated with prednisone (20 mg twice daily), a second-generation H1 antihistamine (fexofenadine 180 mg twice daily or loratidine 10 mg twice daily), an H2 receptor blocker (famotidine 20 mg twice daily or ranitidine 150 mg twice daily), and montelukast (20 mg twice daily) starting 4 days before and continuing throughout RI. Compliance was monitored only by questioning the patient. On the morning of RI, an additional dose of their nonsedating H1 antihistamine and of montelukast (20 mg) were given. The RI extracts used are shown in eTable 1, and the protocol is outlined in eTable 2. Serum was obtained for mature and total tryptase measurements just before the procedure (pre) and either 2 hours after the last immunotherapy dose or 1 to 2 hours after a reaction had begun (post). Frozen samples were thawed and subjected to total tryptase (Phadia ImmunoCAP, Uppsala, Sweden) and mature tryptase (enzyme-linked immunosorbent assay) as described.² Blood pressure, pulse rate, oximetry, and forced expiratory volume in 1 second (FEV₁) were monitored before each injection, 2 hours after the last injection, and as clinically indicated. Injections of increasing allergen amounts were given at 45- to 60-minute intervals. Three nonreactors underwent only 1 day of RI because of personal time constraints. All reactors went on to receive maintenance IT without complications. To be considered a reactor in this study, hypersensitivity symptoms must have occurred before 2 hours after the last dose of allergen during each day of RI.

eTable 1. Immunotherapy Extracts

Ingredient	Amount (cc)	Concentration	Manufacturer
Reactor A			
Extract 1			
Mixed grass (MG)*	1.8	20T Pnu/cc	Alk Abelló
English plantain (EP)*	1.0	10T Pnu/cc	Alk Abelló
Nettle	0.5	1:20 w/v	Hollister-Stier
Alder	1.2	1:20 w/v	Greer
Diluent**	0.5		Alk Abelló
Extract 2			
House dust (HD)*	1.0	10T Pnu/cc	Alk Abelló
Dust mite mix (DMM)	2.0	10,000 Au/cc	Greer
Hollister-Stier dog	1.0	1:100 w/v	Hollister-Stier
Diluent**	1.0		Alk Abelló
Reactor B			
Extract 1			
Mixed grass*	1.6	20T Pnu/cc	Alk Abelló
Mixed trees (MT)*	2.0	10T Pnu/cc	Alk Abelló
Birch*	0.3	10T Pnu/cc	Alk Abelló
English Plantain	0.2	1:10 w/v	Greer
Nettle	0.3	1:20 w/v	Hollister-Stier
Alder	0.2	1:10 w/v	Greer
Cedar	0.3	1:10 w/v	Greer
HZNT	0.1	1:10 w/v	Greer
Extract 2			
House dust*	1.0	10T Pnu/cc	Alk Abelló
Aspergillus	0.4	1:10 w/v	Greer
Fusarium	0.2	1:10 w/v	Hollister-Stier
Cladosporium	0.4	1:20 w/v	Greer
Penicillium	0.2	1:10 w/v	Greer
Pullularia	0.2	1:20 w/v	Greer
Epicoccum	0.4	1:10 w/v	Hollister-Stier
D. farinae	1.7	5000 Au/cc	Greer
CAT (hair)	0.5	10T BAu/cc	Greer
Reactor C			
Extract 1			
Mixed grass*	1.7	20T Pnu/cc	Alk Abelló
Mixed trees*	2.0	10T Pnu/cc	Alk Abelló
Alder*	0.5	10T Pnu/cc	Alk Abelló
Birch*	0.7	10T Pnu/cc	Alk Abelló
Alder	0.1	1:10 w/v	Greer
Extract 2			
House dust*	2.0	10T Pnu/cc	Alk Abelló
English plantain*	0.9	10T Pnu/cc	Alk Abelló
Nettle	0.3	1:20 w/v	Hollister-Stier
Mixed weeds (3)	0.5	1:10 w/v	Greer
J. Cedar (red cedar – <i>juniperus virginiana</i>)	0.3	1:10 w/v	Greer
Cat (hair)	0.1	10T BAu/cc	Greer
Hollister-Stier dog	0.9	1:100 w/v	Hollister-Stier
Extract 3			
Alternaria	0.4	1:20 w/v	Greer
Helminthosporium	0.4	1:10 w/v	Greer
Penicillium	0.7	1:10 w/v	Greer
ALK <i>D farina</i>	0.8	10T Pnu/cc	Alk Abelló
ALK <i>D pteronyssinus</i>	1.5	10T Pnu/cc	Alk Abelló
Diluent**	1.2		Alk Abelló

eTable 1. (Continued) Immunotherapy Extracts

Ingredient	Amount (cc)	Concentration	Manufacturer
Reactor D			
Extract 1			
Mixed grasses*	1.5	20T Pnu/cc	Alk Abelló
Mixed trees*	2.0	10T Pnu/cc	Alk Abelló
English plantain	0.2	1:10 w/v	Greer
Nettle	0.3	1:20 w/v	Hollister-Stier
Mixed weeds (3)	0.3	1:10 w/v	Greer
Alder	0.2	1:10 w/v	Greer
Birch	0.2	1:10 w/v	Greer
Red cedar	0.3	1:10 w/v	Greer
Extract 2			
House dust*	2.2	10T Pnu/cc	Alk Abelló
Alternaria	0.3	1:20 w/v	Greer
Aspergillus	0.4	1:10 w/v	Greer
Cladosporium	0.6	1:20 w/v	Greer
Penicillium	0.4	1:10 w/v	Greer
Dust mite mix	0.6	5,000 Au/cc	Greer
CAT (hair)	0.1	10T BAu/cc	Greer
Hollister-Stier dog	0.4	1:100 w/v	Hollister-Stier
Reactor E			
Extract 1			
English plantain*	1.1	10T Pnu/cc	Alk Abelló
Alder*	1.5	10T Pnu/cc	Alk Abelló
Birch*	1.5	10T Pnu/cc	Alk Abelló
Mixed grass*	0.3	100T Pnu/cc	Alk Abelló
Alder	0.1	1:10 w/v	Greer
Red cedar	0.5	1:10 w/v	Greer
Extract 2			
House dust*	2.0	10T Pnu/cc	Alk Abelló
Dust mite mix	3.0	5,000 Au	Greer
Control F			
Extract 1			
House dust*	1.3	10T Pnu/cc	Alk Abelló
Alk. dust mite mix	2.0	10T Au/cc	Alk Abelló
Hollister-Stier dog	0.7	1:100 w/v	Hollister-Stier
Diluent**	1.0		Alk Abelló
Extract 2			
Fusarium	0.7	1:10 w/v	Hollister-Stier
Helminthosporium	0.7	1:10 w/v	Greer
Cladosporium	0.7	1:20 w/v	Greer
Penicillium	1.1	1:10 w/v	Greer
Trichophyton	0.5	1:10 w/v	Hollister-Stier
Diluent**	1.3		Alk Abelló
Control G			
Extract 1			
Mixed grass*	1.4	20T Pnu/cc	Alk Abelló
Mixed trees*	2.3	10T Pnu/cc	Alk Abelló
English plantain	0.2	1:10 w/v	Greer
Nettle	0.3	1:20 w/v	Hollister-Stier
Alder	0.3	1:10 w/v	Greer
Birch	0.2	1:10 w/v	Greer
Red cedar	0.3	1:10 w/v	Greer
Extract 2			
House dust*	2.1	10T Pnu/cc	Alk Abelló
Mixed molds	2.0	1:10 w/v	Greer/Hollister-Stier
Dust mite mix	0.5	5,000 Au	Greer
Hollister-Stier dog	0.4	1:100 w/v	Hollister-Stier

eTable 1. (Continued) Immunotherapy Extracts

Ingredient	Amount (cc)	Concentration	Manufacturer
Control H			
Extract 1			
Mixed grass*	1.9	20T Pnu/cc	Alk Abelló
English plantain*	1.0	20T Pnu/cc	Alk Abelló
Lambs quarters	0.2	1:20 w/v	Greer
Nettle	0.3	1:20 w/v	Hollister-Stier
Mixed weeds (3)	1.5	1:10 w/v	Greer
Russian thistle (RT)	0.1	1:20 w/v	Greer
Extract 2			
Mixed trees*	3.2	10T Pnu/cc	Alk Abelló
Birch*	1.0	10T Pnu/cc	Alk Abelló
Alder	0.4	1:10 w/v	Greer
Red cedar	0.4	1:10 w/v	Greer
House dust*	1.5	10T Pnu/cc	Alk Abelló
Cat (hair)	0.4	10T BAu/cc	Greer
Hollister-Stier dog	0.7	1:100 w/v	Hollister-Stier
Dust mite mix	2.4	5000 Au	Greer
Control I			
Extract 1			
Mixed grasses*	1.5	20T Pnu/cc	Alk Abelló
Mixed trees*	1.9	10T Pnu/cc	Alk Abelló
English plantain	0.3	1:10 w/v	Greer
Nettle	0.3	1:20 w/v	Hollister-Stier
MW (3)	0.3	1:10 w/v	Greer
Alder	0.4	1:10 w/v	Greer
Birch	0.3	1:10 w/v	Greer
Extract 2			
House dust*	2.0	10T Pnu/cc	Alk Abelló
Penicillium	0.5	1:10 w/v	Greer
Pullularia	0.5	1:20 w/v	Greer
Dust mite mix	1.2	5000 Au	Greer
Cat	0.2	10T BAu/cc	Greer
Diluent	0.6		Alk Abelló
Control 5			
Extract 1			
House dust*	1.4	10T Pnu/cc	Alk Abelló
Alk. dust mite mix	2.2	10T Au/cc	Alk Abelló
Hollister-Stier dog	0.9	1:100 w/v	Hollister-Stier
Diluent**	0.5		Alk Abelló
Extract 2			
Aspergillus	0.8	1:10 w/v	Greer
Helminthosporium	0.6	1:10 w/v	Greer
Cladosporium	0.8	1:20 w/v	Greer
Penicillium	0.8	1:10 w/v	Greer
Pullularia	0.8	1:20 w/v	Greer
Diluent**	1.2		Alk Abelló
Control J			
Extract 1			
House dust*	1.5	10T Pnu/cc	Alk Abelló
Alk dust mite mix	2.3	10T Au/cc	Alk Abelló
Diluent**	1.2		Alk Abelló
Extract 2			
Aspergillus	0.8	1:10 w/v	Greer
Cladosporium	0.8	1:20 w/v	Greer

eTable 1. (Continued) Immunotherapy Extracts

Ingredient	Amount (cc)	Concentration	Manufacturer
Penicillium	0.8	1:10 w/v	Greer
Pullularia	0.3	1:20 w/v	Greer
Epicoccum	0.4	1:10 w/v	Hollister-Stier
Diluent**	1.9		Alk Abelló
Control K			
Extract 1			
Alk. D. pternyssinus	3.0	10T Au/cc	Alk Abelló
Diluent*	2.0		Alk Abelló
Extract 2			
Aspergillus	0.8	1:10 w/v	Greer
Helminthosporium	0.3	1:10 w/v	Greer
Cladosporium	0.6	1:20 w/v	Greer
Penicillium	0.8	1:10 w/v	Greer
Pullularia	0.7	1:20 w/v	Greer
Diluent*	1.8		Alk Abelló
Control L			
Extract 1			
Mixed trees*	2.8	10T Pnu/cc	Alk Abelló
Mixed grasses*	0.3	100T BAu/cc	Alk Abelló
English plantain	0.4	1:10 w/v	Greer
Nettle	0.4	1:20 w/v	Hollister-Stier
Alder	0.4	1:10 w/v	Greer
Birch	0.3	1:10 w/v	Greer
Red cedar	0.4	1:10 w/v	Greer
Extract 2			
House dust*	1.4	10T Pnu/cc	Alk Abelló
Alternaria	0.3	1:20 w/v	Greer
Aspergillus	0.5	1:10 w/v	Greer
Helminthosporium	0.4	1:10 w/v	Greer
Penicillium	0.4	1:10 w/v	Greer
Dust mite mix	1.7	5,000 Au/cc	Greer
Cat	0.1	10T BAu/cc	Greer
Trichophyton	0.2	1:10 w/v	Hollister-Stier
Control M			
Extract 1			
Birch	1.6	10T Pnu/cc	Alk Abelló
Red cedar	0.7	1:10 w/v	Greer
Cat	0.5	10T BAu/cc	Greer
Hollister-Stier dog	0.2	1:100 w/v	Hollister-Stier
Diluent*	2.0		Alk Abelló
Extract 2			
House dust**	1.5	10T	Alk Abelló
Penicillium	0.5	1:10 w/v	Greer
Pullularia	0.5	1:20 w/v	Greer
Dust mite mix	2.5	5000 Au	Greer
Control N			
Extract 1			
Mixed grass*	1.6	20T Pnu/cc	Alk Abelló
Mixed trees*	1.9	10T Pnu/cc	Alk Abelló
Birch	0.5	10T Pnu/cc	Alk Abelló
English plantain	0.3	1:10 w/v	Greer
Nettle	0.3	1:20 w/v	Hollister-Stier
Alder	0.2	1:10 w/v	Greer
Red cedar	0.2	1:10 w/v	Greer

eTable 1. (Continued) Immunotherapy Extracts

Ingredient	Amount (cc)	Concentration	Manufacturer
Extract 2			
House dust*	1.3	10T Pnu/cc	Alk Abelló
Aspergillus	0.5	1:10 w/v	Greer
Penicillium	0.5	1:10 w/v	Greer
Dust mite mix	1.3	5000 Au/cc	Greer
Cat	0.1	10T BAu/cc	Greer
Hollister-Stier dog	0.8	1:100 w/v	Hollister-Stier
Diluent**	0.5		Alk Abelló

* Alum precipitated.

** Diluent is an albumin saline solution with phenol.

eTable 2. Rush Immunotherapy Dosage Steps

Dilution of extracts	Injection (ml) ^a
1:100	0.1, 0.4, 0.8
1:10	0.1, 0.4, 0.7
Undiluted	0.07, 0.15, 0.30, 0.4, 0.5

^a Injections are given at 45-minute intervals from diluted vials and 60-minute intervals from undiluted vials.

eTable 3. Pre- and Post-Mature and Total Tryptase Levels in Reactors Versus Non Reactors

	Mature Tryptase		Total Tryptase		Symptoms and Signs
	Pre	Post	Pre	Post	
Reactors					
A d1	<1	<1	4.1	11	Itching, edema (neck, chest, ears)
A d2	^a	4.5	^a	25	Itching, edema (neck, chin, ears)
B d2	<1	<1	2.9	9	Flushing, nausea
C d1	<1	<1	5.4	13	Flushing, nausea
C d2	<1	<1	6.9	9.9	Flushing
D d2	<1	<1	4.8	23	Lip itching, flushing, urticaria
E d2	<1	<1	4.5	9.3	Mild flushing
Nonreactors					
F	<1	<1	1.7	2.2	None
G	<1	<1	3.6	3.8	None
H	<1	<1	5.2	8.4	None
I	<1	<1	1.9	2	None
J	<1	<1	3.9	2.7	None
K	<1	<1	3.1	3	None
L	<1	<1	3.4	2.8	None
M	<1	<1	<1	<1	None
N	<1	<1	2.8	2.6	None
O	<1	<1	1.6	4.6	Urticaria (following day)

^a For calculations, the d1 Pre tryptase value was used for d2 because no blood was drawn at the beginning of d2.