also suggest that early initiation of PEG-ADA should be considered for ADA-deficient patients suffering from PAP, particularly when rapid hematopoietic stem cell transplantation cannot be performed. Our study also has several significant limitations. While we focused on AMs, future studies are needed to assess the role of pneumocytes and other lung cell abnormalities in the development of PAP in ADA deficiency. In addition, although we found that phagocytosis of IgG-coated beads by ADA-deficient AMs was normal, surfactant uptake was not directly determined. Moreover, AM function differs between humans and mice; hence, further studies with AMs isolated from ADA-deficient patients or developed from induced pleuri-potent stem cells of ADA-deficient patients will be required.

In conclusion, we establish here that ADA-KO mice develop PAP, in association with adenosine, surfactant, and AM abnormalities, and that early restoration of blood ADA activity can prevent PAP and early lethality in ADA-deficient mice.

We thank Pascal Djiadeu for his excellent technical assistance.

Rupreet Dhanju, MSc[a,b]
Weixian Min, MSc[a]
Cameron Ackerley, PhD[c]
Lorand Cimpean, DVM[d]
Nades Palaniyar, MSc, PhD[e]
Chaim M. Roifman, MD, FRCPC, FCACB[d]
Eyal Grunebaum, MD[a,d]

From [a]the Developmental & Stem Cell Biology Program, Hospital for Sick Children, and [b]the Faculty of Medicine, Institute of Medical Science, University of Toronto; [c]the Department of Pathobiology and Laboratory Medicine, Hospital for Sick Children, University of Toronto; [d]the Division of Immunology and Allergy, Hospital for Sick Children, University of Toronto; and [e]the Physiology & Experimental Medicine Program, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. E-mail: eyal.grunebaum@sickkids.ca.

This work was funded by grants to E.G. from the Canadian Immunodeficiency Society and the Jeffrey Modell Foundation. R.D. was supported in part by a graduate scholarship grant from the Canadian Institute for Health Research. L.C. was supported by the Canadian Center for Primary Immunodeficiency.

Disclosure of potential conflict of interest: R. Dhanju has been supported by a Frederick Banting and Charles Best Canada Graduate Scholarships – Master's Award. N. Palaniyar and E. Grunebaum have received research support from the Canadian Institute for Health Research. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Grunebaum E, Cutz E, Roifman CM. Pulmonary alveolar proteinosis in patients with adenosine deaminase deficiency. J Allergy Clin Immunol 2012;129:1588-93.
2. Carey B, Trapnell BC. The molecular basis of pulmonary alveolar proteinosis. Clin Immunol 2010;135:223-35.
3. Blackburn MR, Aldrich M, Volmer JB, Chen W, Zhong H, Kelly S, et al. The use of enzyme therapy to regulate the metabolic and phenotypic consequences of adenosine deaminase deficiency in mice: differential impact on pulmonary and immunologic abnormalities. J Biol Chem 2000;275:32114-21.
4. Blackburn MR, Volmer JB, Thrasher JL, Zhong H, Crosby JR, Lee JJ, et al. Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation, and airway obstruction. J Exp Med 2000;192:159-70.
5. Chunn JL, Molina JG, Mi T, Xia Y, Kellems RE, Blackburn MR. Adenosine-dependent pulmonary fibrosis in adenosine deaminase-deficient mice. J Immunol 2005;175:1937-46.
6. Maygarden SJ, Iacocca MV, Funkhouser WK, Novotny DB. Pulmonary alveolar proteinosis: a spectrum of cytologic, histochemical, and ultrastructural findings in bronchoalveolar lavage fluid. Diagn Cytopathol 2001;24:389-95.
7. Sauer AV, Brigida I, Carriglio N, Aiuti A. Autoimmune dysregulation and purine metabolism in adenosine deaminase deficiency. Front Immunol 2012;3:265.
8. Apasov SG, Blackburn MR, Kellems RE, Smith PT, Sitkovsky MV. Adenosine deaminase deficiency increases thymic apoptosis and causes defective T cell receptor signaling. J Clin Invest 2001;108:131-41.
9. Mohsenin A, Mi T, Xia Y, Kellems RE, Chen JF, Blackburn MR. Genetic removal of the A2A adenosine receptor enhances pulmonary inflammation, mucin production, and angiogenesis in adenosine deaminase-deficient mice. Am J Physiol Lung Cell Mol Physiol 2007;293:L753-61.

Available online January 15, 2014.
http://dx.doi.org/10.1016/j.jaci.2013.11.029

Mendelian inheritance of elevated serum tryptase associated with atopy and connective tissue abnormalities

To the Editor:

The cause of allergic disease is generally considered a complex polygenic process highly influenced by environmental exposures, with mast cells playing a major effector role. Genome-wide association studies and candidate gene approaches have produced an array of allelic variants that impart risk for allergic diatheses.[E1] Although identification of monogenic allergic disease and inherited mast cell disorders has remained less common, several heritable mutations have been identified that help illuminate some pathophysiologic mechanisms underlying the development of atopy in the general population. These include serine peptidase inhibitor, Kazal type 5 *(SPINK5)* mutations in patients with Netherton syndrome[1]; dominant negative signal transducer and activator of transcription 3 *(STAT3)* mutations in patients with a form of hyper-IgE syndrome[2]; and phospholipase C, gamma 2 *(PLCG2)* mutations in patients with a form of familial cold urticaria.[3] Within the mast cell compartment, both germline and somatic mutations in *KIT* have been identified in patients with rare familial presentations of mastocytosis.[4,5]

While seeking to characterize patients with unique, inherited allergic phenotypes among patients referred for evaluation of severe allergic skin, airway, or gastrointestinal disease or systemic mastocytosis (SM), we identified 9 atopic subjects with persistent increases in serum basal tryptase levels in the absence of evidence for a clonal mast cell disorder. Thorough clinical evaluations were undertaken, total and fractionated serum tryptase levels were obtained, *in vitro* basophil activation was assayed, and bone marrow biopsies were performed in 5 of these index patients. For a complete description, see the Methods section in this article's Online Repository at www.jacionline.org. Increased basal serum tryptase levels were found to segregate with distinct clinical features, and it was noted that multiple family members of each index patient shared elements of this phenotype. Serum tryptase levels were obtained in all available family members, and an autosomal dominant inheritance pattern of increased basal total serum tryptase levels was revealed in all 9 families (Fig 1). The mean basal total serum tryptase level measured during a resting state was 21.6 $\pm$ 1.4 ng/mL among subjects with inherited tryptasemia (n = 33). Five family members' tryptase levels were unable to be obtained; these data were excluded from statistical analysis but are included with data from unaffected family members in Table E1 in this article's Online Repository at www.jacionline.org. Tryptase fractionation in a subset of 18 affected patients from 8 of 9 families further revealed that mature tryptase in serum was undetectable (<1 ng/mL, see Table E2 in this article's Online Repository at www.jacionline.org).

Prominent among features segregating with basal serum tryptase level increases were symptoms consistent with chronic and episodic mast cell degranulation, with 26 of 33 subjects

Pedigrees, ages, and clinical phenotypes of affected individuals

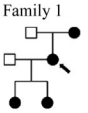
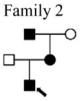
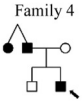
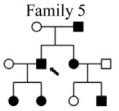
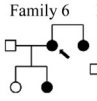
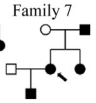
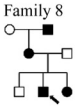
	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9
									
Ages	8, 10, 44, 70	11, 39, 69	6, 10,13, 41	21, 55, 55	17, 22, 23, 48, 57, 78	24, 43, 50	2, 26, 29, 53	12, 20, 44, 68	9, 46
Symptoms *No. affected*									
Cutaneous	**4**	**1**	**4**	**3**	**5**	**2**	**2**	**2**	**2**
Flushing	1	-	2	3	4	1	1	1	-
Urticaria	4	-	3	-	5	2	2	1	1
Angioedema	-	-	1	-	3	-	-	1	-
Pruritis	4	1	2	1	5	1	1	-	2
Connective Tissue	**3**	**3**	**4**	**3**	**0**	**2**	**3**	**3**	**2**
Hypermobility	2	1	4	1	-	2	3	2	-
Retained PrimaryTeeth	-	3	-	1	-	-	-	1	-
Arched Palate	1	1	2	-	-	1	1	-	2
Bone/Vascular abnormality[1]	2	-	-	3	-	-	1	2	-
Atopy	**4**	**3**	**4**	**3**	**6**	**3**	**2**	**4**	**2**
Anaphylaxis	-	2	1	2(1†)	3	-	1	1†	-
Eczema	3	1	3	-	3	-	1	2	2
Asthma	4	2	2	2	4	1	2	1	1
Environmental Allergy	4	2	2	2	4	3	1	3	2
Food Allergy	3	2	1	1	4	-	1	1	-
Drug Allergy	3	1	2[5]	1	5	2	2	1	-
Gastrointestinal	**4**	**3**	**4**	**3**	**5**	**2**	**3**	**4**	**0**
Episodic Pain	2	-	2	3	3	2	3	2	-
IBS/Episodic Diarrhea	1	1[4]	3	-	4	2	2	1	-
GERD	4	1	3	2	3	1	2	2	-
Eosinophilic Esophagitis	1	2	-	1	-	-	-	-	-
Failure to Thrive in Infancy	1	-	3	-	-	-	-	-	-
Neuropsychiatric	**4**	**1**	**3**	**2**	**3**	**2**	**4**	**3**	**2**
Dysautonomia[2]	4	-	3	-	2	1	-	-	-
Anxiety/Depression	4	1	-	1	2	-	-	2	-
Behavioral Disorder	2	1	2	-	1	1	1	-	-
Chronic Pain[3]	2	-	1	2	2	1	3	1	-
Developmental Delay	1	1	-	-	-	-	1	-	2

FIG 1. Pedigrees, ages, and clinical phenotypes of affected subjects. *GERD,* Gastroesophageal reflux disease; *IBS,* irritable bowel syndrome. †Hymenoptera hypersensitivity.[1]Abormalities include pectus excavatum, plagiocephaly/brachycephaly, torticollis, femoral anteversion, tibial torsion, congenital mitral valve insufficiency, tortuous/ectatic intracranial vessels, and scoliosis.[2]Principally manifesting as postural orthostatic tachycardic syndrome.[3]Arthralgia, fibromyalgia, and vulvodynia.[4]Encopresis.[5]Immediate hypersensitivity reaction to radiocontrast media in 1 patient.

reporting episodic urticaria, flushing, and/or cramping abdominal pain frequently associated with urgency, diarrhea, or both. These symptoms could be unprovoked or triggered by heat, exercise, vibration, emotional stress, nonspecific foods, or minor physical trauma. A history of anaphylaxis was reported in 10 of 33 subjects from 6 different families, with 7 occurring following exposure to foods, 2 caused by insect stings, and another without an identified trigger.

Additional triggers in 3 subjects with food-mediated anaphylaxis included stress/heat/exercise (n = 2) and anesthesia (n = 1, Fig 1). One subject (family 1) with a normal serum tryptase level (6.6 ng/mL) had a history of anaphylaxis; this reaction was due to Hymenoptera envenomation (see Table E1).

Gastrointestinal manifestations, whether chronic or episodic, were another prominent feature seen in 28 of 33 subjects with increased tryptase levels; these included eosinophilic gastrointestinal disease, gastroesophageal reflux disease, tenesmus, fecal urgency, irritable bowel syndrome, and diarrhea. Atopic symptoms were present in 31 of 33 tryptasemic subjects, with environmental allergies and asthma being reported among 28 of 33 affected subjects. Connective tissue abnormalities were present in 23 of 33 subjects with increased tryptase levels from 8 of 9 families, and chronic musculoskeletal pain was present among 11 of 33 tryptasemic subjects from 6 of 9 families. Autonomic dysfunction, manifesting as postural orthostatic tachycardia syndrome, was reported in 10 of 33 affected subjects, and a neuropsychiatric diagnosis was present in 17 of 33 affected subjects (Fig 1). Each of these 5 clusters of clinical characteristics (cutaneous, connective tissue, gastrointestinal, atopic, and neuropsychiatric) were independently and significantly associated with increased tryptase levels (Fig 2, *A*). For additional clinical characterization of the families, see the Results section in this article's Online Repository at www.jacionline.org.

Because some of the reported symptoms in tryptasemic subjects could have been consistent with the familial presentation of a clonal mast cell disorder, index patients in 5 of 9 families underwent bone marrow biopsy. A significant increase in mast cell numbers was observed ($P < .05$; mean, 9.6 cells/high-power field [hpf]; range, 4-19.4 cells/hpf) compared with that seen in healthy volunteers (mean, 2.5 cells/hpf; range, 1-5 cells/hpf; see Fig E1 in this article's Online Repository at www.jacionline.org). However, none of the patients met the World Health Organization established criteria for a diagnosis of SM or monoclonal mast cell activation.[E2] Mast cell aggregates were not present, aberrant expression of CD2/CD25 was absent, and *KIT* D816V mutation test results were negative in all 5 patients. Spindled-shaped mast cells (>25%) were observed in a single patient. In the 4 families in which a bone marrow biopsy was not performed, the clinical phenotype aligned with the cohort in which SM was excluded.