

Pulmonary alveolar proteinosis in patients with adenosine deaminase deficiency

Eyal Grunebaum, MD,^{a,b} Ernest Cutz, MD,^c and Chaim M. Roifman, MD^a Toronto, Ontario, Canada

Background: Inherited defects in the function of adenosine deaminase (ADA) cause severe combined immunodeficiency (SCID) and affect many other cells and tissues.

Objectives: We sought to characterize the frequency and features of pulmonary alveolar proteinosis (PAP) in patients with ADA deficiency.

Methods: Clinical and laboratory features of all patients with SCID caused by ADA deficiency in a single center were analyzed. Bronchoalveolar lavage (BAL) fluid and lung biopsy specimens were stained with hematoxylin and eosin and periodic acid–Schiff, visualized by means of electron microscopy, and studied for associated infections. As a control group, BAL fluid and biopsy specimens from 22 patients with SCID caused by other genetic abnormalities were similarly assessed.

Results: Among 16 consecutive patients with ADA deficiency, 7 had BAL fluid containing periodic acid–Schiff–positive surfactant-like material with macrophages engulfing degenerating lamellar bodies and/or lung biopsy specimens with alveolar spaces filled with homogeneous granular eosinophilic material and large macrophages. The lung pathology was typical of PAP. Identification of various pathogens coincided with PAP in 3 of these patients. We have diagnosed PAP among patients with ADA deficiency more commonly in the last 10 years than previously ($P = .05$), likely reflecting increased awareness of this condition. There were no significant differences in the clinical or immunologic characteristics between patients with ADA deficiency with or without PAP. Similar findings of PAP were not found among patients with SCID caused by other genetic abnormalities ($P = .001$). ADA coupled to polyethylene glycol or allogeneic hematopoietic stem cell transplantation rapidly corrected this pulmonary complication. PAP seems to have contributed to the death of only 1 patient with ADA deficiency.

Conclusions: ADA deficiency predisposes to the development of PAP, which could be reversed after enzyme replacement or transplantation. (J Allergy Clin Immunol 2012;■■■:■■■–■■■.)

Key words: Adenosine deaminase, immune, deficiency, pulmonary, alveolar, proteinosis

Abbreviations used

ADA: Adenosine deaminase
BAL: Bronchoalveolar lavage
HSCT: Hematopoietic stem cell transplantation
PAP: Pulmonary alveolar proteinosis
PAS: Periodic acid–Schiff
PEG-ADA: Adenosine deaminase coupled to polyethylene glycol
SCID: Severe combined immunodeficiency

Inherited defects in the adenosine deaminase (ADA) enzyme lead to severe abnormalities in the development and function of T, B, and natural killer lymphocytes, resulting in increased susceptibility to bacterial, viral, and opportunistic infections. Similar to other causes of severe combined immunodeficiency (SCID), defects in ADA are fatal without rapid intervention. Several treatment options are currently available for patients with ADA deficiency, including allogeneic hematopoietic stem cell transplantation (HSCT), enzyme replacement therapy with bovine ADA coupled to polyethylene glycol (PEG-ADA), and gene therapy.¹

ADA catalyses the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. ADA deficiency results in increased concentrations of adenosine and deoxyadenosine in the extracellular and intracellular compartments, which can be phosphorylated to ATP and dATP. The changes in concentrations of these adenine metabolites, particularly the phosphorylated derivatives in patients with ADA deficiency, are thought to be toxic to many cells and tissues, resulting in neurodevelopmental, liver, bone, and bone marrow defects, as well as neutrophil and macrophage dysfunction.^{2–6} Adenosine is also an important mediator of inflammation and fluid homeostasis in the lungs.^{7,8} Therefore adenosine receptor antagonists are being investigated for their putative beneficial effects on capillary leak and cell trafficking in the lungs of patients with several conditions, including asthma.^{7–9}

Little is known about the effects of ADA deficiency on the patient's lungs. We reported lung pathology reminiscent of the findings described among patients with pulmonary alveolar proteinosis (PAP) in 1 patient with ADA deficiency.¹⁰ PAP is a rare disorder with an estimated incidence of 0.36 cases per million population, which is caused by the aberrant accumulation of surfactant-derived components in the lungs, resulting in varying degrees of respiratory difficulties.¹¹ In human subjects the gold standard for diagnosis of PAP are histologic and electron microscopic features detected in samples from bronchoalveolar lavage (BAL) fluid or lung biopsy specimens.¹² The histologic features of PAP include abundant large foamy alveolar macrophages, filling of alveoli, and distal bronchioles with acellular granular amorphous surfactant-derived material that is periodic acid–Schiff (PAS) positive and diastase resistant,¹¹ whereas ultrastructural

From ^athe Division of Allergy and Clinical Immunology, ^bDevelopmental and Stem Cell Biology, and ^cthe Division of Pathology, Department of Pediatric Laboratory Medicine, Hospital for Sick Children and the University of Toronto.

Disclosure of potential conflict of interest: The authors declare they have no relevant conflicts of interest.

Received for publication July 28, 2011; revised January 18, 2012; accepted for publication February 6, 2012.

Corresponding author: Eyal Grunebaum, MD, Division of Immunology and Allergy, Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada. E-mail: Eyal.grunebaum@sickkids.ca.

0091-6749/\$36.00

© 2012 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2012.02.003

TABLE I. Clinical and genetic characteristics of patients with ADA deficiency with PAP

Patient no.	Age at presentation	Presenting features	Age at ADA deficiency diagnosis	Defects in ADA gene sequence (and putative consequences)
1	0.5 mo	IP	0.5 mo	c.955_959delGAAGA and c.478+1G>A (splice site) and heterozygous K80R
2	4 mo	Failure to thrive, neurodevelopmental abnormalities, IP	5 mo	Homozygous for c.424C>T (R142X)
3	3 mo	Failure to thrive, neurodevelopmental abnormalities, IP	5 mo	ND
4	2 mo	Failure to thrive, pneumonia, IP, neurodevelopmental abnormalities, disseminated CMV	3 mo	c.608delG and c.948C>G (S290W)
5	1 mo	IP	1 mo	c.955_959delGAAGA and c.646G>A (G216A) and heterozygous K80R
6	1.5 mo	Diarrhea, erythroderma, IP	4 mo	c.955_959delGAAGA and c.424C>T (R142X)
7	1 mo	Urinary tract infection, IP	2 mo	c.424C>T (R142X) and c.955_959delGAAGA and heterozygous K80R

CMV, Cytomegalovirus; IP, interstitial pneumonitis; ND, not done.

analysis often demonstrates abnormal intra-alveolar “onion skin”-shaped lamellar bodies.¹³

PAP can be classified as congenital, primary, or secondary. The congenital type is caused by rare defects in surfactant production or macrophage function. The latter have been attributed to inherited mutations in GM-CSF and its receptor. Primary PAP, which accounts for nearly 90% of cases, often results from autoantibodies that neutralize GM-CSF signaling in alveolar macrophages. Secondary PAP occurs in patients with systemic inflammatory diseases and malignancies, as well as in association with lung infections, including cytomegalovirus, *Mycobacterium tuberculosis*, *Nocardia* species, and *Pneumocystis jiroveci*.^{11,13}

Here we characterize the lung abnormalities in a cohort of patients with ADA deficiency and identify among them a high frequency of PAP-like abnormalities that improved rapidly after PEG-ADA or allogeneic HSCT.

METHODS

All patients identified with SCID caused by ADA deficiency at the Hospital for Sick Children, Toronto, Ontario, Canada, since 1985 were reviewed. The diagnosis of ADA deficiency was based on the absence or near absence of ($\leq 2\%$) ADA activity in red blood cells and confirmed by using molecular analysis. Clinical and laboratory information was collected retrospectively from patients' medical records and censored June 30, 2011. Normal laboratory values for age were determined in accordance with previous reports.^{10,14} BAL fluid and lung biopsy specimens were analyzed for the presence of bacteria; viruses, including herpes viruses, adenoviruses, and metapneumovirus; and fungi, as well as *P jiroveci* pneumonia and mycobacterial species. Samples were stained with hematoxylin and eosin and PAS. When PAS staining was pale or inconclusive, immunostaining for specific surfactant protein A was performed. Samples were also analyzed by using electron microscopy.

As a control group, BAL fluid and lung biopsy specimens obtained before HSCT from patients with non-ADA SCID who had lung disease without an identified microbe were also analyzed. All lung samples were analyzed by a pathologist (E.C.) unaware of the genetic causes of the immunodeficiency. The institute's Research Ethics Board approved the study. Statistical analysis was performed with Fisher exact and Student *t* tests.

RESULTS

Clinical and laboratory characteristics of patients with ADA deficiency

Among the 16 patients with SCID given a diagnosis of ADA deficiency during the 26.5 years of the study period, 7 (43.8%)

had PAP. In the most recent 10 years, PAP was recognized among 6 of the 9 patients with ADA deficiency compared with 1 of 7 patients given a diagnosis in the preceding 16.5 years ($P = .05$). Two of the 9 patients who did not have PAP compared with none of the 7 patients given a diagnosis of PAP ($P = .30$) were identified at birth because of prior affected siblings. The remaining 14 patients with ADA deficiency presented during the first 6 months of life with recurrent invasive infections, interstitial pneumonitis, failure to thrive, or neurodevelopmental abnormalities (Table I). Patients had no clinical evidence of autoimmunity, although 1 patient among those with PAP and another among the patients without PAP had Omenn syndrome.¹⁵ The age of first clinical symptoms among patients with ADA deficiency with PAP (mean, 1.5 months; range, 0.5–4 months) did not differ ($P = .83$) from that of patients without PAP (mean, 1.5 months; range, 0–6 months). Similarly, the age of diagnosis of ADA deficiency among patients with PAP (mean, 3 months; range, 0.5–5 months) and those without PAP (mean, 6 months; range, 0–18 months) were not significantly different ($P = .28$).

ADA activity was low (≤ 2 nmol/min/mL) in all patients, regardless of the identification of PAP, except for one in whom activity could not be determined because of prior blood transfusions. Sequencing of the ADA gene was available for the 7 patients with PAP (Table I) and 7 of the 9 patients without PAP. The c.955_959 deletion was identified among 4 patients with PAP compared with 1 patient without PAP ($P = .13$), and c.424 C>T (putatively causing R142X) was found in 3 patients with PAP and 1 without PAP. In contrast, homozygous c.7C>T (putatively causing Q3X) was identified among 4 patients with ADA deficiency without PAP but not in patients with PAP ($P = .04$).

At the time of diagnosis of ADA deficiency, leukopenia was present in 5 of 7 patients with PAP and 8 of 9 patients without PAP, and neutropenia was found in 1 patient with PAP (Table II) and 2 patients without PAP. Severe lymphopenia ($<0.7 \times 10^9$ lymphocytes/mL) with markedly reduced CD3⁺ T-cell numbers was recorded in all 16 patients with ADA deficiency. Serum IgG levels were reduced in 3 patients with PAP and 5 patients without PAP ($P = .22$). The remaining patients with ADA deficiency, all 6 months of age or less, had normal IgG levels, likely reflecting maternal-fetal IgG transfer.

By the time PAP was identified, 6 of the 7 patients with ADA deficiency had received broad-spectrum antibiotics (Table III), which is similar to the use of antibiotics in 7 of the 9 patients

TABLE II. Laboratory evaluations at the time of ADA deficiency diagnosis

Patient no.	Leukocytes ($\times 10^9/L$)	Neutrophils ($\times 10^9/L$)	Monocytes ($\times 10^9/L$)	Lymphocytes ($\times 10^9/L$)	CD3 ⁺ T cells ($\times 10^9/L$)	IgG*
1	3.6	2.9	0.1	0.2	0.02	5.0
2	8.5	7.2	1.2	0.1	0.00	3.6
3	4.8	4.4	0.6	0.3	0.23	0.5
4	9.0	6.7	1.9	0.3	0.14	0.7
5	3.5	2.9	0.3	0.2	0.03	3.2
6	4.5	2.5	0.1	0.2	0.09	1.4
7	2.4	1.0	0.8	0.1	0.01	3.0

Boldface data are abnormal for age.

*Serum IgG levels before immunoglobulin replacement therapy (in grams per liter); normal age levels are 2.3 to 14.1 g/L.

TABLE III. Characteristics of PAP in patients with ADA deficiency

Patient no.	Treatment before PAP diagnosis	Mono* ($\times 10^9/L$)	T cells† ($\times 10^9/L$)	BAL/Bx (age)	Lung infection
1	BSA	0.2	0.02	Bx (1 mo)	No
2	BSA, chemotherapy	0.7	0	Bx (9 mo)	No
3	BSA	0.6	0.23	BAL (5 m)	Yes (<i>M catarrhalis</i>)
4	BSA, ganciclovir	0.3	0.14	Bx (4 mo)	Yes (CMV)
5	none	0.3	0.03	BAL (1 mo)	No
6	BSA	0.1	0.09	BAL (5 mo)	No
7	BSA	0.7	0.01	BAL (2 mo)	Yes (adenovirus)

Boldface data are abnormal for age.

BSA, Broad-spectrum antibiotics; Bx, lung biopsy; CMV, cytomegalovirus; NA, not applicable.

*Blood monocytes ($\times 10^9/L$) at the time of BAL/Bx.

†Blood CD3⁺ cells ($\times 10^9/L$) at the time of BAL/Bx.

without PAP ($P = .60$). In addition, before the diagnosis of PAP, 1 patient was treated for disseminated cytomegalovirus infection with ganciclovir, whereas another patient (patient 2) had received busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg) in preparation for HSCT.

We also assessed the numbers of monocytes and CD3⁺ T cells in patients with ADA deficiency at the time PAP was identified (Table III). Less than normal monocyte numbers were found among 2 of the patients with PAP but not in patients without PAP ($P = .18$). The mean and SD values of CD3⁺ T-cell numbers in patients with PAP ($0.74 \pm 0.85 \times 10^9$ cells/mL) were not different ($P = .10$) than the numbers seen in patients without PAP ($0.192 \pm 0.157 \times 10^9$ cells/mL).

Lung findings in patients with ADA deficiency with PAP

BAL fluid, lung biopsy specimens, or both identified PAP within 1 month of the diagnosis of ADA deficiency in 6 patients, whereas in another patient with ADA deficiency (patient 2), lung biopsy identified PAP 4 months after allogeneic HSCT (Table III). BAL fluid in 4 patients with PAP was described as milky and contained PAS-positive surfactant-like material with macrophages engulfing degenerating lamellar bodies. Lung biopsy specimens from 3 additional patients with PAP revealed alveolar spaces filled with homogeneous granular eosinophilic material (Fig 1, A), whereas staining for surfactant protein A demonstrated that the intra-alveolar material was consistent with surfactant (Fig 1, B). Electron microscopy further showed that the intra-alveolar material was composed of concentric lamellar bodies typical of surfactant (Fig 1, C), whereas higher magnification of these lamellar bodies was characteristic of phospholipids (Fig 1, D). Type 1 and 2 pneumocytes appeared normal.

Identification of *Moraxella catarrhalis*, cytomegalovirus, or adenovirus in the lungs coincided with PAP in 3 different patients with ADA deficiency with PAP (Table III). *Staphylococcus aureus*, *Streptococcus pneumoniae*, and respiratory syncytial virus were all found in a single patient without PAP. Cumulatively, microbial isolates were identified in 3 of 7 patients with PAP and in 3 of 9 patients without PAP ($P = .37$). An additional patient given a diagnosis at birth of ADA deficiency because of a previous family member had BAL at 1 month of age because of interstitial pneumonitis, with no evidence of PAP or infectious cause.

To determine whether PAP was more common in patients with ADA deficiency, we assessed BAL fluid and lung biopsy specimens from 22 patients with SCID caused by other genetic defects, including 12 patients with IL-2 receptor γ (*IL2RG*) deficiency, 2 with Janus kinase 3 (*JAK3*) deficiency, 3 with recombination-activating gene 1/2 (*RAG1/2*) mutations, 2 with ζ chain-associated protein 70 kD (*ZAP70*) deficiency, and 1 patient each with MHC class II deficiency, RNA component of mitochondrial RNA processing deficiency, and IL-7 receptor α (*IL7RA*) deficiency. Remarkably, the rate of PAP among patients with ADA deficiency (7/16 patients) was significantly higher ($P = .001$) than in patients with SCID caused by other genetic defects (0/22 patients).

Management and outcome of patients with ADA deficiency with PAP

Management options used for patients with ADA deficiency with PAP included PEG-ADA enzyme replacement, allogeneic HSCT, or both (Table IV). In all but 1 patient (patient 2), there was rapid (usually 2-4 weeks) complete clinical and radiologic resolution of PAP after these treatments, well before any effects on lymphocyte number or function became evident. Moreover, repeat

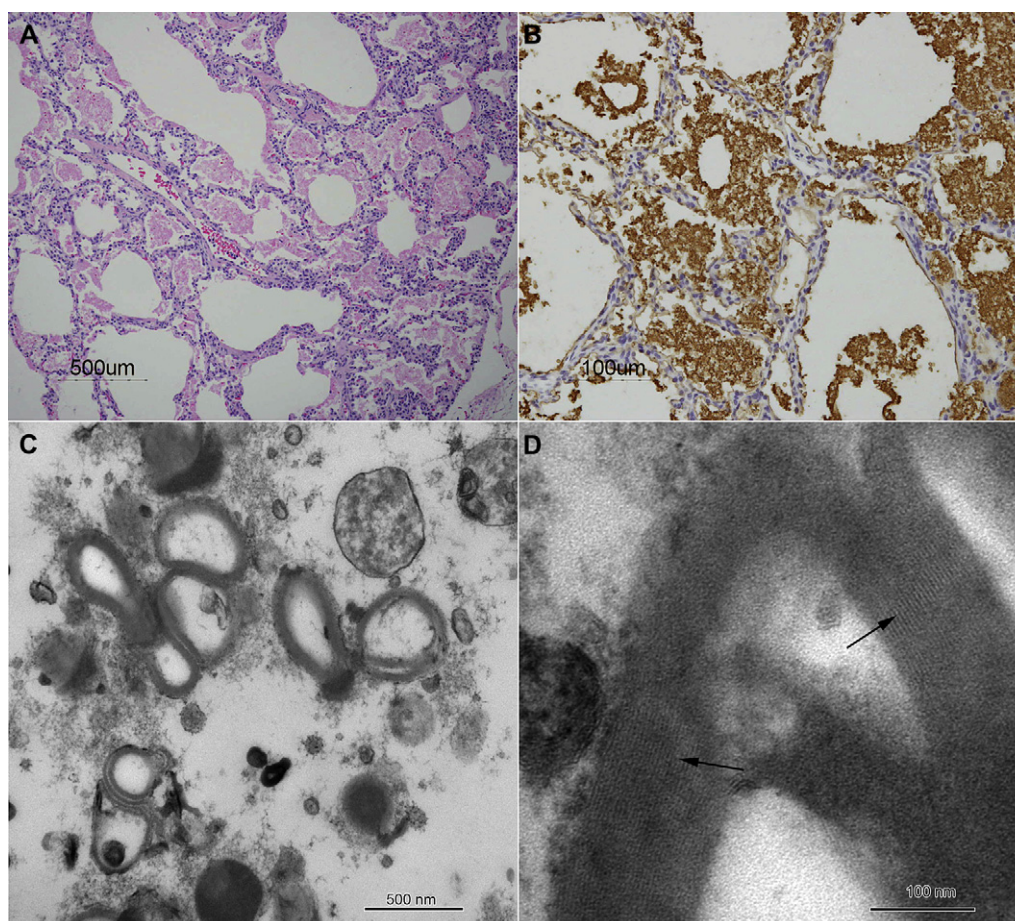


FIG 1. Lung sample from a patient with ADA deficiency. **A**, Hematoxylin and eosin staining demonstrating alveolar spaces filled with homogeneous eosinophilic material. **B**, Immunostaining with antibodies to surfactant protein A demonstrating positive immunoperoxidase reaction (*brown*) of intra-alveolar material. **C**, Electron micrograph demonstrating alveolar surfactant composed of concentric lamellar bodies. **D**, Electron micrograph at higher magnification showing a lamellar body with characteristic phospholipids.

lung biopsy in patient 1, as well as autopsy in patient 6 (who died accidentally), demonstrated histologic resolution of PAP.

Only 2 (28.6%) of 7 patients with ADA deficiency with PAP are long-term survivors, which is similar ($P = .14$) to the 6 of 9 patients without PAP who survived. PAP contributed directly to the death of 1 patient (patient 2). Immune reconstitution among the surviving 2 patients with ADA deficiency is suboptimal because both have reduced numbers of B cells and 1 requires intravenous immunoglobulin replacement (Table IV). CD3⁺ T-cell and CD56⁺ natural killer cell numbers were also reduced in 1 patient.

DISCUSSION

ADA deficiency is among the most common forms of SCID, and its effects on cellular and humoral immunity have been extensively investigated.¹⁶ In addition, we and others have studied various neurologic, liver, skeletal, and bone marrow abnormalities in patients with ADA deficiency.²⁻⁵ Here we report for the first time that patients with ADA deficiency are also prone to lung disease that is indistinguishable from PAP. The increased frequency of PAP among patients with ADA-deficient SCID but not other SCID types, together with the detection of PAP in the

absence of microbial isolates among 4 patients with PAP, all suggest direct contribution of ADA deficiency to the development of PAP. The latter hypothesis is also supported by the rapid resolution of the lung abnormalities after ADA enzyme replacement, even before immune reconstitution. Hence we conclude that PAP is a specific and previously unappreciated consequence of ADA deficiency.

In an attempt to identify potential causes for the development of PAP in patients with ADA deficiency, we compared the clinical features, including ages of presentation and ages of diagnosis, as well as immunologic and metabolic features, among patients with ADA deficiency with PAP versus those of patients without PAP. We did not find significant differences between the groups. However, among patients with PAP, there was a trend for a higher frequency of the c.955_959delGAAGA mutation, which is expected to cause unstable mRNA¹⁷ and c.424 C>T (R142X), which are expected to significantly disrupt the function of ADA. Further studies on a larger number of patients will be required to determine whether specific genetic abnormalities in patients with ADA contribute to the development of PAP.

Although we did not assess autoantibodies to GM-CSF or its receptor, the hypogammaglobulinemia found frequently in our

TABLE IV. Management and outcome of patients with ADA deficiency with PAP

Patient no.	ADA deficiency treatment (age)	PAP resolution	Patient status, age, major complications, cause of death	CD3 (× 10 ⁹ /L)	CD19 (× 10 ⁹ /L)	CD56 (× 10 ⁹ /L)	IgG (g/L)*
1	HSCT (2 mo)	Yes	Died at 300 mo, demyelinating brain disease	NA	NA	NA	NA
2	HSCT (8 mo)	No	Died at 11 mo, graft-versus-host disease and PAP	NA	NA	NA	NA
3	HSCT (11 mo)	Yes	Died at 11.5 mo, gastrointestinal bleed	NA	NA	NA	NA
4	None	Yes	Died at 4.5 mo, severe neurodevelopmental defects	NA	NA	NA	NA
5	PEG-ADA (1.5 mo) followed by gene therapy (at 16 mo)	Yes	Alive at 65 mo, lipoblastoma	1.19	0.10	0.02	IVIG
6	HSCT (7 mo)	Yes	Died at 13 mo, severe neurodevelopmental defects, accidental death	NA	NA	NA	NA
7	HSCT (2.5 mo)	Yes	Alive at 25 mo, none	1.37	0.09	0.14	4.7

Boldface data are abnormal for age.

IVIG, Intravenous immunoglobulin; NA, not applicable.

*Normal IgG levels for age are 7.2 to 15.8 g/L for patients 1 and 3; 5.7 to 14.7 g/L for patients 7, 9, and 11; 5.0 to 14.6 g/L for patient 13; and 4.5 to 14.3 g/L for patients 15 and 16.

patients argues against a role of autoantibodies in the development of PAP in patients with ADA deficiency. Moreover, the lack of monocytopenia in peripheral blood of patients with ADA deficiency with PAP suggests appropriate monocyte proliferation and maturation from bone marrow progenitors, which are also dependent on GM-CSF signaling,¹⁸ although direct evaluation of GM-CSF and its receptor are still required.

Interestingly, 2- to 3-week-old ADA-deficient mice, even when kept in a pathogen-free environment, have severe respiratory disease that can be rapidly corrected with PEG-ADA treatment or neonatal HSCT. The lungs of ADA-deficient mice contained increased numbers of enlarged and foamy macrophages with a prominent increase in the size of alveolar spaces and thickened smooth muscle in the pulmonary blood vessels. In addition, excessive PAS-positive mucus accumulated in the alveoli and bronchi of these mice, although the nature of this substance and the cause for its deposition were not elucidated, and hence correlation with PAP was not established.¹⁹ Further studies with the ADA-deficient mice indicated that excess adenine nucleotides and abnormal signaling through various adenosine receptors in the lungs resulted in altered expression and secretions of several cytokines, chemokines, and metalloproteinases, including IL-4 and IL-13, eotaxin 2, CXCL1, and matrix metalloproteinases 9 and 12.²⁰⁻²² In addition, IL-6 was recently found to contribute to the inflammation, alveolar air-space enlargement, and mucus metaplasia seen in the lungs of ADA-deficient mice, which could be attenuated by pharmacologic neutralization of IL-6.²³ Future studies will help determine whether similar mechanisms also contribute to the lung abnormalities in patients with ADA deficiency and the role of adenosine receptor inhibitors or IL-6 neutralization in treating PAP in these patients.

Various infections have been associated previously with PAP; however, these have not commonly included *M catarrhalis*, cytomegalovirus, or adenovirus, which coincided with the identification of PAP among our patients with ADA deficiency.²⁴ Thus the role of these microbes in the development of PAP remains unclear. Moreover, impaired surfactant production has been shown to directly impede innate immunity, as well as the function of neutrophils and macrophages,^{25,26} suggesting that PAP in patients

with ADA deficiency might further increase the susceptibility to lung infections.

Regardless of the cause of PAP in patients with ADA deficiency, increased awareness, early identification, and rapid treatment of ADA deficiency-associated PAP with enzyme replacement or HSCT might reverse this potentially fatal lung condition.

Key message

- Pulmonary alveolar proteinosis is frequent among patients with ADA deficiency.

REFERENCES

1. Gaspar HB, Aiuti A, Porta F, Candotti F, Hershfield MS, Notarangelo LD. How I treat ADA deficiency. *Blood* 2009;114:3524-32.
2. Nofech-Mozes Y, Blaser SI, Kobayashi J, Grunebaum E, Roifman CM. Neurologic abnormalities in patients with adenosine deaminase deficiency. *Pediatr Neurol* 2007;37:218-21.
3. Somech R, Lai YH, Grunebaum E, Le Saux N, Cutz E, Roifman CM. Polyethylene glycol-modified adenosine deaminase improved lung disease but not liver disease in partial adenosine deaminase deficiency. *J Allergy Clin Immunol* 2009;124:848-50.
4. Sauer AV, Mrak E, Hernandez RJ, Zacchi E, Cavani F, Casiraghi M, et al. ADA-deficient SCID is associated with a specific microenvironment and bone phenotype characterized by RANKL/OPG imbalance and osteoblast insufficiency. *Blood* 2009;114:3216-26.
5. Sokolic R, Maric I, Kesserwan C, Garabedian E, Hanson IC, Dodds M, et al. Myeloid dysplasia and bone marrow hypocellularity in adenosine deaminase-deficient severe combined immune deficiency. *Blood* 2011;118:2688-94.
6. Csóka B, Selmeczy Z, Koscsó B, Németh ZH, Pachter P, Murray PJ, et al. Adenosine promotes alternative macrophage activation via A2A and A2B receptors. *FASEB J* 2012;26:376-86.
7. Schepp CP, Reutershan J. Bench-to-bedside review: adenosine receptors-promising targets in acute lung injury? *Crit Care* 2008;12:226.
8. Caruso M, Varani K, Tringali G, Polosa R. Adenosine and adenosine receptors: their contribution to airway inflammation and therapeutic potential in asthma. *Curr Med Chem* 2009;16:3875-85.
9. Kreindler JL, Shapiro SD. Lung turns to AA (adenosine analogues) to dry out. *Nat Med* 2007;13:406-8.
10. Grunebaum E, Mazzolari E, Porta F, Dalleria D, Atkinson A, Reid B, et al. Bone marrow transplantation for severe combined immune deficiency. *JAMA* 2006;295:508-18.
11. Doerschuk CM. Pulmonary alveolar proteinosis—is host defense awry? *N Engl J Med* 2007;356:547-9.

12. Burkhater A, Silverman JF, Hopkins MB 3rd, Geisinger KR. Bronchoalveolar lavage cytology in pulmonary alveolar proteinosis. *Am J Clin Pathol* 1996;106:504-10.
13. Carey B, Trapnell BC. The molecular basis of pulmonary alveolar proteinosis. *Clin Immunol* 2010;135:223-35.
14. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* 2003;112:973-80.
15. Roifman CM, Zhang J, Atkinson A, Grunebaum E, Mandel K. Adenosine deaminase deficiency can present with features of Omenn syndrome. *J Allergy Clin Immunol* 2008;121:1056-8.
16. Hershfield MS. New insights into adenosine-receptor-mediated immunosuppression and the role of adenosine in causing the immunodeficiency associated with adenosine deaminase deficiency. *Eur J Immunol* 2005;35:25-30.
17. Hirschhorn R, Chen AS, Israni A, Yang DR, Huie ML. Two new mutations at the adenosine deaminase (ADA) locus (Q254X and del nt1050-54) unusual for not being missense mutations. *Hum Mutat* 1993;2:320-3.
18. Barreda DR, Hanington PC, Belosevic M. Regulation of myeloid development and function by colony stimulating factors. *Dev Comp Immunol*, 2004 function by colony stimulating factors. *Dev Comp Immunol* 2004;28:509-54.
19. 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.
20. Mohsenin A, Blackburn MR. Adenosine signaling in asthma and chronic obstructive pulmonary disease. *Curr Opin Pulm Med* 2006;12:54-9.
21. Mohsenin A, Burdick MD, Molina JG, Keane MP, Blackburn MR. Enhanced CXCL1 production and angiogenesis in adenosine-mediated lung disease. *FASEB J* 2007;21:1026-36.
22. Sun CX, Zhong H, Mohsenin A, Morschl E, Chunn JL, Molina JG, et al. Role of A2B adenosine receptor signaling in adenosine-dependent pulmonary inflammation and injury. *J Clin Invest* 2006;116:2173-82.
23. Pedroza M, Schneider DJ, Karmouty-Quintana H, Coote J, Shaw S, Corrigan R, et al. Interleukin-6 contributes to inflammation and remodeling in a model of adenosine mediated lung injury. *PLoS One* 2011;6:e22667.
24. Seymour JF. Extra-pulmonary aspects of acquired pulmonary alveolar proteinosis as predicted by granulocyte-macrophage colony-stimulating factor-deficient mice. *Respirology* 2006;11(suppl):S16-22.
25. Sender V, Moulakakis C, Stamme C. Pulmonary surfactant protein A enhances endolysosomal trafficking in alveolar macrophages through regulation of Rab7. *J Immunol* 2011;186:2397-411.
26. Doua DN, Jackson R, Grasmann H, Palaniyar N. Innate immune collectin surfactant protein D simultaneously binds both neutrophil extracellular traps and carbohydrate ligands and promotes bacterial trapping. *J Immunol* 2011;187:1856-65.