



Recent advances in understanding and managing adenosine deaminase and purine nucleoside phosphorylase deficiencies

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Purpose of the review

To review the recent advances in the understanding and management of the immune and nonimmune effects of inherited adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiencies.

Recent findings

Abnormal thymocyte development and peripheral T-cell activation in ADA-deficient and PNP-deficient patients cause increased susceptibility to infections and immune dysregulation. The impaired purine homeostasis also damages many other cell types and tissues. Animal studies suggest that defects in surfactant metabolism by alveolar macrophages cause the pulmonary alveolar proteinosis commonly seen in ADA-deficient infants, while toxicity of purine metabolites to cerebellar Purkinje cells may lead to the ataxia frequently observed in PNP deficiency. Patients' outcome with current treatments including enzyme replacement and stem cell transplantations are inferior to those achieved in most severe immunodeficiency conditions. New strategies, including intracellular enzyme replacement, gene therapy and innovative protocols for stem cell transplantations hold great promise for improved outcomes in ADA and PNP deficiency. Moreover, newborn screening and early diagnosis will allow prompt application of these novel treatment strategies, further improving survival and reducing morbidity.

Summary

Better understanding of the complex immune and nonimmune effects of ADA and PNP deficiency holds great promise for improved patients' outcome.

Keywords

adenosine deaminase, immunodeficiency, purine nucleoside phosphorylase, systemic

INTRODUCTION

Adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) are consecutive enzymes important for purine degradation and salvage (Fig. 1). ADA catalyzes the irreversible deamination of 2'-deoxyadenosine (dAdo) and adenosine while PNP catalyzes the reversible phosphorolysis of inosine, guanosine, deoxyinosine and deoxyguanosine (dGuo). Tight control of ADA and PNP substrates, including their phosphorylated products ATP, dATP, GTP and dGTP, is critical for many biological processes including cell signaling, energy production and DNA formation. Indeed, accumulation of dAdo and dGuo is toxic to lymphocytes [1,2], while elevated dGTP and dATP interfere with DNA repair [3–5].

Inherited autosomal recessive defects in the function of ADA and PNP were identified in the 1970's as causing severe immune abnormalities

[6,7]. ADA deficiency is particularly common among certain communities such as the American First Nations, Mennonites and Somali [8,9], and is estimated to account for 20–25% of all severe combined immune deficiency (SCID) in North America. PNP deficiency is less frequent, and a recent review identified 67 patients with PNP deficiency worldwide [10]. Increased awareness, better techniques to assess immune function as well as availability of ex-vivo and animal models that closely recapitulate

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KEY POINTS

- ADA and PNP deficiency are associated with diverse immune and nonimmune abnormalities.
- Understanding and appreciating the complex effects of ADA and PNP deficiency has significantly advanced the diagnosis and management options for affected patients.
- New strategies, including intracellular enzyme replacement, gene therapy and innovative protocols for hematopoietic stem cell transplantation hold great promise for improving the outcome of ADA and PNP-deficient patients.

the human disease have significantly advanced the understanding and consequently the management options for ADA-deficient and PNP-deficient patients.

IMMUNE ABNORMALITIES IN ADENOSINE DEAMINASE AND PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY

Patients with defects that completely abrogate ADA activity typically present in the first months of life with recurrent bacterial, viral, fungal or opportunistic infections. Analysis of lymphocyte subpopulations in these patients often reveals progressive

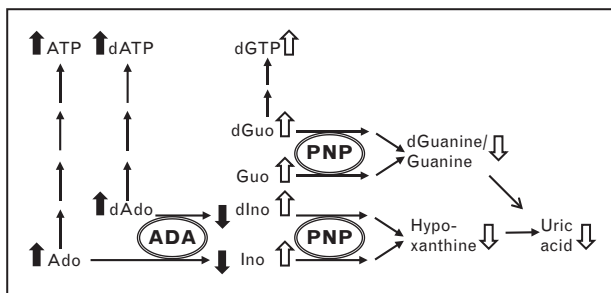


FIGURE 1. Biochemical effects of adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiency. ADA deaminates adenosine (Ado) and deoxyadenosine (dAdo) to inosine (Ino) and deoxyinosine (dIno), respectively. Ino and dIno as well as guanosine (Guo) and deoxyguanosine (dGuo) can undergo phosphorolysis by PNP. Alternatively, Ado/dAdo or Gua/dGuo can be phosphorylated to ATP/dATP or GTP/dGTP, respectively. ADA deficiency (block arrows) cause marked increase in the concentrations of Ado, dAdo, ATP and dATP and prevents formation of Ino and dIno. PNP deficiency (open arrows) cause marked accumulation of guanosine and deoxyguanosine (dGuo) and subsequently dGTP (through the action of mitochondrial dGuo kinase). PNP deficiency also causes reduced guanine, hypoxanthine and uric acid.

and eventually complete or near complete absence of T, B and NK lymphocytes ($T^{-}B^{-}NK^{-}$ SCID). In contrast, patients with some residual ADA activity (approximately 5% of normal) might have a normal number of lymphocytes and be identified later, even at adulthood [11–13]. PNP-deficient patients often suffer from infections in their first decade of life, which are caused by opportunistic pathogens including JC virus, *Aspergillus fumigatus* and *Mycobacterium* [14,15]. Although there is a progressive decline in the number of T lymphocytes, B and NK cells are often spared ($T^{-}B^{+}NK^{+}$ SCID). Nevertheless, few PNP-deficient patients have also been identified with severe infections in their first year of life and absent B cells [16[■],17]. Thus, while many infections afflicting ADA-deficient and PNP-deficient patients represent the particular sensitivity of T cells to disrupted purine homeostasis, B cells might also be affected in both conditions [17,18].

Recent studies have shed additional light on the mechanisms leading to abnormal T-cell development and function. Analysis of the thymus of an ADA-deficient patient revealed severe atrophy as well as loss of cortex-medulla demarcation [19]. Immature thymus epithelial cells were abundant while AutoImmune Regulator expressing mature thymus epithelial cells were absent. Dendritic cells were severely depleted while macrophage-like cells were increased, possibly because of the need to remove apoptotic thymocytes. Another group investigated causes for the impaired responses of ADA-deficient T cells to stimulation. $CD4^{+}$ T cells from ADA-deficient patients had severely compromised TCR/CD28-driven activation, including phosphorylation of Zeta Associated Protein-70, Ca^{2+} flux and ERK1/2 signaling, resulting in impaired proliferation and cytokine production [20]. Hence, diverse mechanisms might account for abnormal function of T cells in ADA deficiency.

Studying the effects of PNP deficiency on T-cell development and function has been more challenging because of limited access to patients' samples as well as the confounding effects of chronic infections and immune-modulating medications. To overcome this obstacle, we recently analyzed T lineage cells from PNP $^{-/-}$ mice, created previously in our lab [2]. Similarly to PNP-deficient patients, these mice display metabolic derangements, which include markedly increased concentrations of dGuo in cells and plasma as well as decreased uric acid in the urine. PNP $^{-/-}$ mice also demonstrate progressive T lineage defect with reduced, albeit not absent responses of T cells to stimulation [21], reminiscent of those observed in PNP-deficient patients [22[■]]. The thymus of PNP $^{-/-}$ mice are small and contain increased $CD4^{-}CD8^{-}$ double negative thymocytes

and significantly decreased numbers of CD4⁺CD8⁺ double positive cells [23]. The double positive thymocytes demonstrate accelerated apoptosis, which is initiated in the mitochondria and induced by dGuo. In contrast, proliferation and diversity of thymocytes from PNP^{-/-} mice, as well as proliferation of double positive-like cells grown *ex vivo* from bone marrow of PNP^{-/-} mice using the OP9-DL1 culture system [24] were normal, suggesting a preferential effect of abnormal purine homeostasis on cells at the late double negative to double positive maturation/selection stages.

Importantly, in addition to the susceptibility to infections, ADA-deficient and PNP-deficient patients often suffer from immune dysregulation leading to diabetes mellitus, autoimmune thyroiditis, hepatitis and autoimmune hematological cytopenia [25–27]. A recent study [28^{***}] demonstrated significantly reduced frequency of CD4⁺CD25⁺FoxP3⁺ regulatory T cells among ADA-deficient patients and that these cells had reduced ability to suppress proliferation of co-cultured autologous responder T cells. Similarly, regulatory T cells from ADA-deficient mice had significantly reduced suppressive activity. Whether regulatory T cell abnormalities are also responsible for Omenn's syndrome that we previously described in ADA-deficient patients [29] or the frequent autoimmunity observed in PNP-deficient patients is still not clear. The autoimmunity observed in ADA deficiency might also be due to defective central and peripheral B cell tolerance. Indeed, new emigrant/transitional and mature naive B cells from ADA-deficient patients were shown to contain excess auto-reactive clones. Moreover, B cell receptor and Toll-like receptor functions in B cells are impaired after ADA inhibition, which might also contribute to B-cell tolerance defects [30^{***}].

NON-IMMUNE ABNORMALITIES IN ADENOSINE DEAMINASE AND PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY

ADA and PNP are expressed in practically all cells, hence it is not surprising that defects in these enzymes causes systemic effects unrelated to the immune deficiency. Here we will focus on recent studies of brain, lung, bone and bone marrow abnormalities associated with ADA and PNP deficiency.

Brain abnormalities associated with adenosine deaminase and purine nucleoside phosphorylase deficiency

ADA-deficient patients may suffer neurological deficits, developmental delay and sensory-neural

hearing loss secondary to infections, autoimmunity or transplantation [31–33]. Additionally, we described hypotonia, head lag, nystagmus, difficulties in focusing gaze, seizures and developmental delay among ADA-deficient infants, which were not explained by infections or medications [34]. Computed tomographic scans and cranial MRI of these patients revealed volume loss as well as basal ganglia and thalamus abnormalities, possibly due to accelerated nerve cell death or altered stimulation of adenosine receptors. Neurological abnormalities, such as spastic paresis that can be confused with cerebral palsy, disequilibrium, ataxia and developmental delay, are even more common in PNP deficiency, affecting more than 50% of patients [35,36]. Although some of these neurological deficits might be attributed to infections or vasculitis, in many patients an etiology cannot be found. Several inborn errors of purine metabolism, including Lesch–Nyhan syndrome and deoxyguanosine kinase deficiency result in severe neurological abnormalities, suggesting that abnormal purine homeostasis might be directly responsible for the neurological defects in PNP deficiency [37]. Therefore, we recently used the PNP^{-/-} mouse model to better understand the effects of PNP deficiency on the brain. PNP^{-/-} mice had normal muscle strength as well as memory and learning abilities; however, they displayed motor defects including frequent falls from a spinning rod and balance beam [38^{*}]. MRI of PNP^{-/-} mice brains revealed significantly smaller than normal corpus callosum, thalamus and cerebellum. Histological analysis of the cerebellum of these mice showed progressive reduction in the number of Purkinje cells. Moreover, Purkinje cells from the cerebellum of PNP^{-/-} mice, grown *ex vivo*, exhibited increased apoptosis. Restoration of PNP function in the brain of PNP^{-/-} mice prevented the neurological abnormalities, confirming that the neurological damage was directly related to the disrupted purine metabolism. Studying ADA^{-/-} mice as well as neuronal-like cells from ADA-deficient and PNP-deficient patients derived induced pleuri-potent stem cells will further help understand the mechanisms responsible for the neurological abnormalities.

Lung abnormalities associated with adenosine deaminase deficiency

Many ADA-deficient patients suffer from severe lung disease, which in a few cases has been attributed to infections and/or immune dysregulation [25]. Yet, in most ADA-deficient patients no respiratory pathogens are isolated [39]. We recently reviewed lung abnormalities among 16 ADA-deficient

patients treated in our center [40[¶]]. Broncho-alveolar lavage and lung biopsies identified accumulation of Periodic Acid Schiff positive, surfactant-like granular material as well as macrophages engulfing lamellar bodies, typical of pulmonary alveolar proteinosis (PAP) in seven patients (43.8%). PAP is often classified as congenital, primary or secondary [41]. The congenital type is caused by rare defects in surfactant production or macrophage function, such as those occurring in patients with mutations in granulocyte-macrophage colony stimulating factor (GM-CSF) pathway. Primary PAP often results from autoantibodies that neutralize GM-CSF signaling in alveolar macrophages, while secondary PAP is associated with systemic inflammatory diseases, malignancies and some lung pathogens, which were not identified in our patients. To confirm that ADA deficiency causes PAP and to better understand the pathogenesis of PAP, we used an ADA^{-/-} mouse model [42]. ADA^{-/-} mice suffer from many of the metabolic, immune and systemic features observed in ADA-deficient patients and develop lethal respiratory disease by 3 weeks of age [43]. We found accumulation of surfactant in the alveoli of ADA^{-/-} mice (Fig. 2). Broncho-alveolar fluid from these mice contained excessive protein, cholesterol and alveolar macrophages, with excessive macrophage apoptosis. Importantly, early restoration of ADA activity in ADA^{-/-} mice prevented the development of PAP, thereby confirming that ADA deficiency directly contributes to PAP formation [44].

Bone abnormalities associated with adenosine deaminase and purine nucleoside phosphorylase deficiency

ADA deficiency causes various skeletal abnormalities in 50% of ADA-deficient patients. To further characterize these changes we recently analyzed chest radiographs performed in the first 6 months of life among nine ADA-deficient patients [45]. In all patients, we found unusual scapular spurring and anterior rib cupping. Follow-up imaging performed 6–12 months after initiation of ADA enzyme replacement therapy or allogeneic hematopoietic stem cell transplantations revealed complete resolution of the bony radiographic changes. Studies of ADA^{-/-} mice identified potential mechanisms responsible for the skeletal abnormalities [46]. These mice displayed low trabecular bone volume and reduced number of trabeculi. Moreover, osteoblast cultures from ADA^{-/-} mice showed significantly reduced proliferation rate, decreased viability and increased apoptosis. Interestingly, Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL)

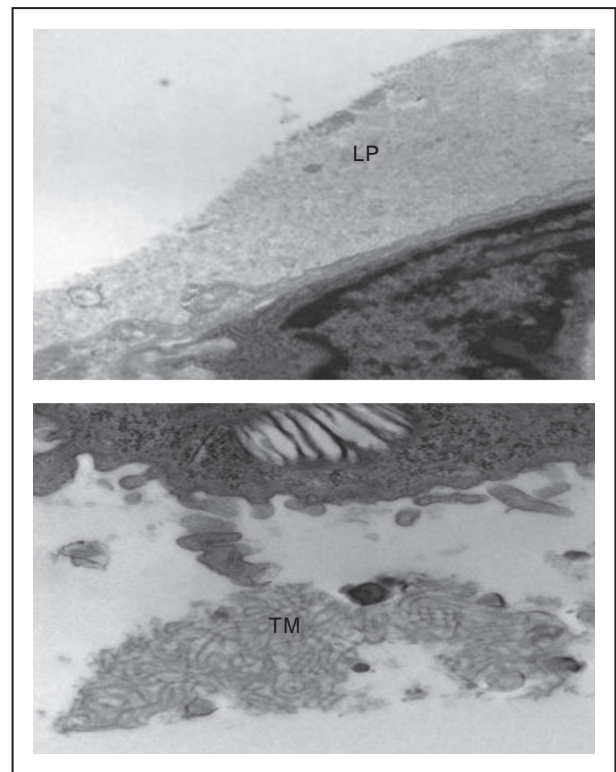


FIGURE 2. Alveolar proteinosis in the lungs of adenosine deaminase (ADA)-deficient mice. Electron microscopy images of lung sections from ADA^{-/-} mice show extensive accumulation of granular lipoproteinaceous (LP) substance in the alveoli (upper panel) and that the substance has a tubular myelin (TM) structure, typical of surfactant (lower panel).

expression levels were significantly reduced in these cells, corresponding with the decreased serum RANKL levels found in ADA^{-/-} mice [46]. Although osteoblasts from ADA-deficient patients were not studied, the significantly decreased serum RANKL levels found in these patients [46] suggests that mechanisms similar to those found in ADA^{-/-} mice account for the patients bone abnormalities.

Neutropenia and bone marrow abnormalities associated with adenosine deaminase and purine nucleoside phosphorylase deficiency

Neutropenia in ADA-deficient and PNP-deficient patients has often been related to autoimmunity or infections [47]. Additionally, we previously described neutropenia and hematopoietic stem cell dysplasia in an 8-year-old PNP-deficient patient as well as increased sensitivity of the patient's bone marrow stroma to ionizing damage [48]. Recently hypocellular bone marrow and mild dysplastic changes of three hematopoietic lineages were also identified in an ADA-deficient patient [49].

Moreover, 13 ADA-deficient patients exhibited various abnormalities in their peripheral neutrophils including prolonged neutropenia, hypogranular and vacuolated neutrophils as well as hyperlobular and pyknotic neutrophils [50]. These patients also had large/giant platelets, as well as atypical eosinophils with cytoplasmic vacuoles, uneven granulation and hyperlobular nuclei. Bone marrow examinations performed in a few ADA-deficient patients revealed myelodysplastic changes. Importantly, there was an inverse relationship between phosphorylated ADA substrates and neutrophil numbers suggesting a primary myelotoxic process [50]. Studies in ADA^{-/-} mice provided additional insight into the potential effects of impaired ADA function on the bone marrow [46]. Fibroblast colony formation assays showed that ADA-deficient bone marrow mesenchymal progenitor cells grew normally, but these cells supported long-term culture initiating colony formation less efficiently than wild-type cells. Proteome profiling of total cytokine and chemokine production from the supernatant of the cultures revealed reduced levels of M-CSF, IL-6, CXCL1/10, and soluble Intra-Cellular Adhesion Molecule 1. Importantly, the percentage of early hematopoietic stem cells was significantly lower in the bone marrow of ADA^{-/-} mice [46], although it is still not clear whether this reflects a primary hematopoietic defect or is secondary to a stroma defect.

MANAGEMENT OF ADENOSINE DEAMINASE AND PURINE NUCLEOSIDE PHOSPHORYLASE DEFICIENCY

In recent years, improved understanding of inherited purine defects complexity has led to significant advances in the management of ADA and PNP deficiency. Moreover, earlier identification of patients, as well as rapid initiation of treatments will be crucial in preventing irreversible toxicity to many organs, including the thymus, brain and lungs.

Newborn screening for adenosine deaminase and purine nucleoside phosphorylase deficiency

Real time quantitative PCR measurement of T-cell receptor excision circles (TREC) in dried blood spots, a surrogate to the number of T cells in peripheral blood, was recently adopted as an efficient newborn screening method for SCID [51]. While this strategy can detect T cells absence at birth, the lymphoid toxicity in some ADA-deficient and PNP-deficient patients manifests later in life, hence these patients might not be identified by TREC screening.

Alternatively, measurement of ADA and PNP enzyme activity by reverse-phase high-performance liquid chromatography or detection of increased concentration of ADA and PNP metabolites by tandem mass spectrometry were proposed as methods to detect purine defects in newborn dried blood spots [52–54]. Indeed, a recent report demonstrated that newborns with partial ADA deficiency would have been detected by mass spectrometry but not by TREC analysis [55[■]].

Allogeneic hematopoietic stem cell transplantations for adenosine deaminase and purine nucleoside phosphorylase deficiency

Traditionally, allogeneic hematopoietic stem cells transplantations have been offered as definitive treatments for ADA- and PNP-deficient patients. Recently, a large multicenter study [56[■]] of 106 ADA-deficient patients who received transplants revealed 67% survival and 20% transplant-related mortality. Transplants using HLA-matched sibling (MSD) donors and HLA-matched family donors (MFD) resulted in approximately 85% survival while usage of haplo-identical (defined as >2 HLA antigen mismatch) donors resulted in only 43% survival. Although MSD/MFD transplantations were performed without conditioning, engraftment was observed in 90% of patients. In contrast, almost all haplo-identical transplantations performed without conditioning failed to engraft. Immune reconstitution, determined by the number of T cells, was also significantly faster following MSD/MFD transplantations than after haplo-identical transplantations. Donor chimerism in T cells was 75–100% for most patients who received MSD/MFD transplants, while approximately half of the patients who received haplo-identical transplants had 0–24% donor chimerism. Following haplo-identical transplantations, patients who had appropriate donor chimerism were primarily those who received myeloablative conditioning. Only 22 patients received transplantations from HLA-matched or mis-matched unrelated donors, with 67% and 29% survival, respectively. The rate of T-cell recovery following transplantations from unrelated donors was also slow although eventually, among the surviving patients, most achieved 75–100% T-cell donor chimerism. Accordingly, for ADA-deficient patients who do not have a MSD or MFD, the European Society for Immunodeficiency recommends continuation of ADA enzyme replacement, if available and effective, or to proceed with gene therapy or matched unrelated donor transplantation [57].

It has been customary to stop PEG-ADA prior to transplanting ADA-deficient patients, thereby providing 'survival advantage' to the ADA-proficient cells, and to avoid cyto-reductive chemotherapy because of the increased sensitivity of ADA-deficient cells to ionizing radiation. However, recent data from gene therapy trials as well as from ADA^{-/-} mice have challenged this approach. Low doses of Busulfan were shown to improve engraftment without excessive toxicity [26], while continuing PEG-ADA improved survival of ADA^{-/-} mice without compromising engraftment of the transplanted gene corrected cells [58[¶]]. Whether this strategy or alternative nonmyeloablative conditioning regimens will also benefit ADA-deficient patients remains to be established.

There are very few reports of transplantation in PNP-deficient patients, possibly reflecting the hesitance to transplant these patients who are often diagnosed beyond infancy and suffer from multiorgan damage including neurological abnormalities. The outcome of transplantation in PNP deficiency has traditionally been poor, possibly for reasons similar to those in ADA deficiency, although there are no formal large studies to answer these important questions. Nevertheless, recent case reports demonstrate that MSD/MFD transplantation at an early age, even without conditioning, might be beneficial and prevent further neurological deterioration [59–61].

Gene therapy for adenosine deaminase and purine nucleoside phosphorylase deficiency

ADA and PNP deficiencies have been among the first human inherited immune defects in which replacement of the faulty gene in hematopoietic stem cells was investigated. Several groups have recently described outcome in more than 25 ADA-deficient patients who received gene therapy [26,57,62[¶]]. Following withdrawal of ADA enzyme replacement and administration of chemotherapy at nonmyeloablative doses, patients were infused with autologous bone marrow cells treated with retroviruses encoding the human ADA gene [63]. Subsequently, T cell numbers increased to above the lower limit of normal in almost 50% of the patients, with polyclonal T-cell repertoires, although TREC remained below normal levels. There was also improved B-cell tolerance [30^{¶¶}], and half of the patients were able to stop intravenous immunoglobulin therapy. The rate of severe infections decreased and no life-threatening opportunistic infections were noted after gene therapy. Importantly, there was no evidence of insertion mutagenesis and malignant transformations that marred gene therapy trials for other

inherited immune defects [64]. The tumors that did develop after gene therapy for ADA deficiency, including dermato-fibrosarcoma and lipoblastoma, were not the result of viral integration and likely reflected abnormal DNA repair or impaired tumor surveillance as patients often exhibited only partial correction of the purine homeostasis and immune reconstitution [65[¶],66]. Currently, several groups are investigating methods to improve ADA gene therapy efficacy and safety and a clinical trial was recently opened in the USA and Europe using self-inactivating lentiviral vectors expressing ADA under control of internal mammalian promoters [62[¶],67]. Additionally, studies in ADA^{-/-} mice suggest that continuation of ADA enzyme replacement therapy might enhance engraftment of gene corrected cells [58[¶]]. Currently, there are no gene therapy trials for PNP-deficient patients, although we previously demonstrated that lentiviruses could efficiently and safely deliver human PNP in PNP^{-/-} mice and improve the mice immune function [68]. Disappointingly, the expression of PNP was only transient and the effect on PNP^{-/-} mice survival was only moderate. Hence, additional preclinical studies are needed before PNP gene therapy can be offered to patients.

Enzyme replacement therapy for adenosine deaminase and purine nucleoside phosphorylase deficiency

The ability of purine metabolites to exit cells through nucleoside transporters has been utilized for many years for enzyme replacement in ADA and PNP deficiency by the use of purine rich red blood cell infusions or polyethylene glycol conjugated-bovine ADA (PEG-ADA) injections [69,70]. However, PEG-ADA often results in only temporary and partial immune reconstitution; hence it is most commonly used for short periods to stabilize patients prior to transplantation. Longer reliance on PEG-ADA treatment has frequently resulted in development of infections, autoimmunity or malignancy at an older age [18,71,72]. PEG-ADA disappointing long-term effects have been attributed to many factors including enzyme inactivation by neutralizing antibodies to the bovine ADA, difficulties in reaching all tissues such as the thymus and need for the enzyme to function inside the cell. Additionally, extra-cellular detoxification with PEG-ADA or red blood cell transfusions might be limited by the dependency on nucleoside transporters (Fig. 3). Lastly, concerns about cost, estimated at more than \$100 000/year per patient and transmission of Bovine spongiform encephalopathy prompted search for alternative replacement

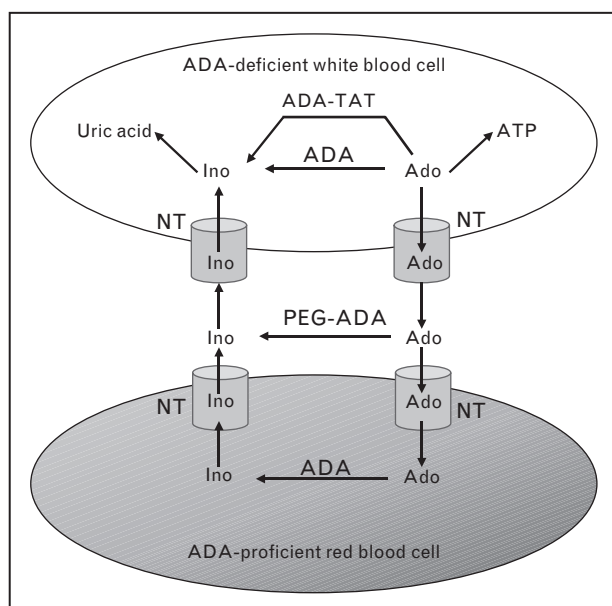


FIGURE 3. Advantage of restoring adenosine deaminase (ADA) within cells versus extra-cellular ADA. Restoring intracellular ADA with ADA-TAT reduces the concentrations of adenosine (Ado) and its phosphorylated product ATP, as well as normalizes inosine (Ino), independently of nucleoside transporters (NT). In contrast, correction of intracellular purine abnormalities with extra-cellular ADA (such as exogenous ADA supplied by PEG-ADA or by ADA-proficient red blood cells) depends on transfer of purines through NT.

options. Sigma–Tau Pharmaceuticals, Inc. has developed a recombinant human ADA conjugated to PEG (EZN-2279), however its safety and efficacy in animal models or human are not yet known.

To address some of the limitations associated with PEG-mediated replacement, we recently combined the human PNP to the 11 essential amino acids of the HIV TAT protein transduction domain, which is capable of transferring proteins across biological membranes [73,74]. Our PNP-TAT recombinant protein, which was expressed and isolated from bacteria, significantly increased PNP enzyme activity in B-lymphocytes from a PNP-deficient patient [75]. Bi-weekly injections of PNP-TAT restored purine degradation in PNP^{−/−} mice and prevented the immune dysfunction and early death of these mice [76]. Moreover, prolonged PNP-TAT treatment normalized immune function and survival of PNP^{−/−} mice for more than 52 weeks. Importantly, PNP-TAT entered the brain of PNP^{−/−} mice and prevented the neurological abnormalities typically seen in these mice [38*]. Our exciting results with TAT-PNP have led us to produce a similar recombinant protein of human ADA fused to TAT, which is currently being evaluated *in vitro* and in ADA^{−/−} mice.

Future management options for adenosine deaminase and purine nucleoside phosphorylase deficiency

Cells apoptosis in ADA and PNP deficiency have been attributed to accumulation of dATP in the cytoplasm and dGTP in the mitochondria, respectively [23,77]. Indeed, inhibition of adenosine kinase and deoxycytidine kinase with 5'-amino-5'-deoxy-adenosine and 2'-deoxycytidine, respectively, prevented the accumulation of dATP and restored differentiation and proliferation of ADA-deficient human thymocytes *ex vivo*, which might also be beneficial for patients [78]. Similarly, prevention of dGTP accumulation or mitochondria-initiated apoptosis might benefit PNP-deficient patients. Additionally, recognizing the contribution of alveolar macrophage defects to the development of PAP in ADA deficiency suggests a potential benefit from granulocyte-macrophage colony stimulating factor.

CONCLUSION

There has been significant progress in understanding the complex immune and nonimmune effects of abnormal purine homeostasis in ADA and PNP deficiency in recent years. These advancements are bound to improve the management of affected patients.

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None.

Conflicts of interest

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