

Deficiency of Adenosine Deaminase 2 (DADA2), an Inherited Cause of Polyarteritis Nodosa and a Mimic of Other Systemic Rheumatologic Disorders

Hasan Hashem¹ · Susan J Kelly² · Nancy J Ganson² · Michael S Hershfield^{2,3}

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Abstract

Purpose of Review A new autoinflammatory disease, deficiency of adenosine deaminase 2 (DADA2), caused by mutations in the *CECR1* gene, was first reported in 2014. This review aims to update progress in defining, treating, and understanding this multi-faceted disorder.

Recent Findings DADA2 was first described in patients with systemic inflammation, mild immune deficiency, and vasculopathy manifested as recurrent stroke or polyarteritis nodosa (PAN). More than 125 patients have now been reported, and the phenotype has expanded to include children and adults presenting primarily with pure red cell aplasia (PRCA), or with antibody deficiency. Age of onset and clinical severity vary widely, even among related patients, and are not clearly related to *CECR1* genotype. Inflammatory features often respond to anti-TNF agents, but marrow failure and severe immune deficiency may require hematopoietic stem cell transplantation.

Summary ADA2 is expressed and secreted by monocytes and macrophages, but its biological function and the pathogenesis of DADA2 are uncertain and will remain an important area of research. Pre-clinical investigation of ADA2 replacement therapy and *CECR1*-directed gene therapy are warranted, but complicated by the absence of a suitable animal model.

Keywords Monogenic autoinflammatory disorder · *CECR1* · Vasculopathy · Polyarteritis nodosa · Stroke · Immune deficiency · Pure red cell aplasia · Macrophage polarization · TNF inhibitor · Hematopoietic stem cell transplant · Adenosine deaminase · Whole-exome sequencing

Introduction

Genome-wide association studies (GWAS) have identified many loci that influence the inflammatory and autoimmune diseases most familiar to rheumatologists, but their diagnosis relies on clinical presentation, pathological findings, and disease-associated autoantibodies. By contrast, gene sequencing is required for unambiguous diagnosis of more than 25 rare monogenic autoinflammatory diseases, which typically lack specific biomarkers [1]. Most of these genes were identified by positional cloning, homozygosity mapping, and candidate gene screening of large pedigrees. In the last few years, whole-exome sequencing (WES) has enabled unbiased discovery of mutated genes in single or small numbers of patients with novel clinical presentations.

In 2014, investigators at the NIH and in Israel independently reported the use of WES to define a new autoinflammatory disorder caused by loss of function mutations in both copies of *CECR1* (cat eye syndrome critical region candidate 1). This 10-exon/28-kb gene on chromosome 22 q11.1 encodes a 511-amino-acid protein, adenosine deaminase 2 (ADA2); the disorder has been named deficiency of ADA2, abbreviated DADA2 [2•, 3•]. ADA2 was discovered 40 years ago in tissues of patients with severe combined immune deficiency (SCID) who lacked ADA1, the major ADA activity in humans, which is encoded by the *ADA* gene on chromosome 20 [4]. ADA1 is a largely intracellular, 41-kDa monomer expressed in all

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✉ Michael S Hershfield
michael.hershfield@dm.duke.edu

¹ King Hussein Cancer Center, Amman 11941, Jordan

² Duke University School of Medicine, Durham, NC 27710, USA

³ Duke University Medical Center, Box 3049, Durham, NC 27710, USA

cells, with highest levels in lymphocytes. ADA2 is a homodimer of 57-kDa subunits; it is expressed mainly in myeloid cells and is secreted by monocytes, macrophages, and dendritic cells [5, 6]. Structurally, the proteins have similar active sites, but ADA2 has additional domains involved in its secretion, dimerization, and binding to a putative receptor [7, 8]. Mice lack a *CECR1* orthologue and do not express ADA2.

The initial report of ADA2 showed that it had much lower affinity for its substrates, adenosine (Ado) and 2'-deoxyadenosine (dAdo), than does ADA1, and is insensitive to a purine analog, EHNA, which strongly inhibits ADA1 [4]. Assays based on this differential inhibition have shown that ADA2 activity is elevated in serum of patients with chronic hepatitis and some infections, including tuberculosis and HIV, as well as from patients with various primary immunodeficiency diseases [9]. Though intriguing, this finding has had little utility, and only in retrospect does it hint at some critical function of ADA2 in humans. Assay of plasma ADA2 activity now provides an inexpensive and rapid means of testing for DADA2.

Clinical Presentation of the First DADA2 Patients

The first two reports of DADA2 described 33 patients with periodic fever, livedoid rash, elevated inflammatory markers, vasculopathy, and mild immune deficiency mainly affecting B cells [2••, 3••]. Vasculopathy was manifested primarily as early-onset lacunar stroke in one study, and as polyarteritis nodosa (PAN) in the other, reflecting the presentation of the index patients. Thirteen different *CECR1* mutations were identified, mostly missense, which mapped to the signal peptide, catalytic, dimerization, and putative receptor binding domains of ADA2. In addition to showing that DADA2 is clinically and genetically heterogeneous, these reports provided important insight into pathogenesis and therapy (for other recent reviews, see [10, 11]).

Of the nine NIH patients, eight had strokes, in most cases, by age 5 years, which were ischemic, hemorrhagic, or both, and on neuroimaging, localized to deep nuclei, midbrain, and brain stem [2••]. The 9th patient had small vessel vasculitis. In addition to livedo racemosa and fever (8/9 patients), other findings in four or five patients each included hepatosplenomegaly, hypogammaglobulinemia (with recurrent bacterial and viral infections in two of four patients), variable lymphopenia, and low serum IgM. All were ANCA-negative. Skin biopsies showed vasculitis of medium-sized arteries. Brain biopsies in two patients showed erythrocyte extravasation without inflammation. Endothelial damage and endothelial cell activation were also found in skin and brain biopsies, along with increased staining for IL-1 β , iNOS, and TNF- α . Plasma levels of these and other cytokines were normal. In several patients, circulating memory B cells

were reduced, and B cells showed increased apoptosis in vitro. The activity of ADA1 in red cells was normal, and there was no elevation in dAdo nucleotides (a hallmark of ADA1 deficiency). There was no sustained clinical response to immunosuppressive therapy, or to anakinra, an inhibitor of IL-1 that is effective in several other autoinflammatory diseases.

Elkan et al. described 24 patients with features of PAN, including 19 from 5 Israeli families of Georgian Jewish origin, who had been followed for many years; 4 German patients from a single family; and 1 Turkish patient (of 14 with PAN in whom *CECR1* was sequenced) [3••]. The patients were negative for ANCA and had histopathologic findings consistent with PAN [12]. Notably, disease severity and age of onset were highly variable, even within families. Four patients had only cutaneous PAN (cPAN) without constitutional symptoms (the oldest presented with leg ulcers at 59 years old), and 13 had severe PAN (digital necrosis, visceral organ injury), which was fatal in 2 cases at 9 m and 31 years of age. Of ten skin biopsies, four showed necrotizing arterial vasculitis and six showed nonspecific leukocytoclastic vasculitis or panniculitis. Among the 19 Israeli patients, all homozygous for the G47R mutation, 18 had cPAN, mainly livedo reticularis; 10 also had visceral PAN, diagnosed by 1 year of age in 6 cases, with stenosis and aneurysms of abdominal arteries, or renal cortical infarcts and hypertension. Neurologic involvement in 8 of these 19 patients was primarily peripheral, but in some cases, brain neuroimaging showed infarcts and ventricular hemorrhage, even in patients who lacked a clinical diagnosis of stroke. The four German siblings and the Turkish patient were compound heterozygotes for four different missense mutations. Of note, 10 of 13 patients with severe PAN had sustained benefit from treatment with anti-TNF agents.

As mice lack a *CECR1* orthologue, the NIH investigators used the zebrafish as a model, showing that transient knock-down of the *cecr1b* gene (orthologous to human *CECR1*) caused intracranial bleeding and marked neutropenia in embryos, which were prevented by providing wild-type, but not mutant, human *CECR1* mRNA [2••]. Neither *CECR1* mRNA nor ADA2 protein could be detected in normal human endothelial cells, suggesting that endothelial damage and vasculopathy in patients might involve macrophages, which secrete ADA2 and play a role in inflammation. Patient-derived monocytes showed normal GM-CSF induced differentiation into M1 (pro-inflammatory) macrophages, but defective M-CSF induced differentiation of into M2 (anti-inflammatory) macrophages. Human dermal endothelial cells co-cultured with patient, but not control monocytes, formed abnormal layers. Together, these findings led to a hypothesis that ADA2 deficiency, by impairing M2 macrophage differentiation, leads to M1 polarization, compromising endothelial integrity and establishing a vicious cycle of vasculopathy and inflammation [2••].

Phenotypic Diversity

Reports describing ~ 125 DADA2 patients have appeared to date (key features are summarized in Table 1). Some patients were screened for *CECR1* mutations because of clinical suspicion, e.g., a family with Sneddon syndrome, a non-inflammatory arteriopathy with onset of livedo reticularis in the second decade and stroke in early adulthood [18•]. In other cases, bi-allelic *CECR1* mutations were found unexpectedly when WES was performed in patients with a different phenotype, e.g., a single patient with HHV8-negative (idiopathic) Castelman disease and another with monocytopenia and *M. avium* complex infection (MonoMAC syndrome), but without mutation in *GATA2* [17•, 31].

The use of WES has led to the discovery of DADA2 in a growing number of patients who present with pure red cell aplasia (PRCA), sometimes occurring with other cytopenias, and with or without overt signs of inflammation or vasculopathy [14•, 28•, 34•]. DADA2 patients with PRCA differ from patients with Diamond Blackfan anemia (DBA), in which PRCA is associated with mutations in genes for ribosomal proteins or *GATA1*. About 70% of typical DBA patients have elevated levels of erythrocyte ADA1 activity, which is not found in PRCA due to DADA2. It is uncertain whether the bone marrow dysfunction associated with DADA2 represents an intrinsic defect or is secondary, possibly due to impaired interaction (perhaps mediated via secreted ADA2) between components of the hematopoietic microenvironment and macrophages or some other myeloid cell type.

Various immunological abnormalities, mainly of the B cell compartment, were observed in the original DADA2 patients. Persistent autoimmunity has been documented in a few patients [15•, 38]. Two reports have now described 11 patients with DADA2 who presented primarily with antibody deficiency and infections, compatible in several cases with a diagnosis of common variable immune deficiency (CVID) [30, 37••]. Median age of these patients at evaluation was 22 years, and it was 8 years at onset of symptoms (Table 1). It has been postulated that the defect in B cell maturation/differentiation might arise from aberrant communication between the innate and adaptive immune systems.

The presence of inflammatory CNS and skin involvement both in DADA2 and in the Aicardi Goutieres syndrome (due to mutations in *SAMHD1*) have led to the discovery of increased expression of interferon genes (“interferon signature”) in peripheral blood of several DADA2 patients; genome-wide overexpression of neutrophil genes was also found in some of these patients [19••, 27, 38]. These findings have led to the proposal that ADA2 may be a regulator of neutrophil activation, and that the vasculopathy in DADA2 patients may be an indirect effect of marked chronic neutrophil activation [19••]. It is unclear how enhanced neutrophil activation, or for

that matter defective M2 macrophage differentiation, might result in selective bone marrow failure in some patients, but PAN in others.

Together, the reports summarized in Table 1 have expanded the phenotype and the number of pathogenic *CECR1* mutations associated with DADA2; they also highlight the variability in disease expression and the lack of a clear relationship between *CECR1* genotype and clinical phenotype. All clinically affected patients have been deficient in circulating ADA2 activity, even when only one disease causing *CECR1* variant can be identified (i.e., by routine methods, but without a search for large deletions). As *CECR1* is highly polymorphic, measurement of plasma ADA2 activity is also useful in excluding DADA2 when DNA sequencing reveals only one pathogenic mutation (alone or in combination with a variant of uncertain significance). Two caveats should be mentioned: some apparently healthy but *CECR1*-mutated and ADA2-deficient individuals have been discovered among first-degree relatives of clinically affected patients, raising difficult questions about the need for and timing of therapy. Conversely, although DADA2 has an autosomal recessive pattern of inheritance, a few carriers with half-normal plasma ADA2 activity have exhibited some clinical features associated with DADA2, raising the possibility of *CECR1* haploinsufficiency, perhaps involving effects of modifier genes.

Therapy

Multiple therapeutic options have been described in case reports and a few case series, but the choice of treatment for an individual patient depends on the predominant symptoms and severity. Immunosuppressive agents have controlled inflammation in some patients, but response is usually transient. Anti-TNF agents were effective in 10 of 13 Israeli patients with severe PAN [3••], and in 12 patients treated at the NIH for a median of 10 months with no new strokes or other serious complications; cPAN improved in these patients, although livedo persisted in some [39]. Nine of ten symptomatic patients in a series from the UK required anti-TNF therapy [26•]. Steroids were ineffective in six Turkish patients with PAN, but two benefitted from anti-TNF therapy [23•]. At an international workshop on DADA2 held in Bethesda, MD, in November 2016, there seemed to be a consensus that anti-TNF agents are a preferred treatment, but as the course of the disease is variable over time, it is not yet clear whether their use will indefinitely prevent the occurrence of strokes or the emergence of PAN. There is also uncertainty about the role of anti-platelet and anticoagulant prophylaxis for stroke, with some indication that it might increase the risk for hemorrhagic events.

Sirolimus, which can reduce M1 macrophage differentiation and IL-6 production, was effective in controlling

Table 1 Reports expanding the phenotype and the number of pathogenic *CECR1* mutations associated with DADA2

Reference	No. of patients (families)	Phenotype	CECR1 mutations (no. of patients with genotype)	Comment
[2••]	9 (8)	Fever (9/9); livedo racemosa (8/9); stroke (8/9); HSM (6/7); documented vasculitis 4/9; PAN 2/9; low serum IgM (5/5); ANA+ (3/9); ANCA+ (0/9); ophthalmologic abnormalities (5/9)	A109D/Y453C G47A/Y453C R169Q/28-kb del G47A/H112Q R169Q/Y453C G47A/Y453C MT/193T G47R/G47R (3) G47R/G47R (19) R169Q/P251L (4) G47V/W264S (1) G47R/R306*	Recurrent stroke predominant presentation of vasculopathy; proposed endothelial injury and vasculopathy due to impaired M2 polarization of macrophages
[3••]	24 (6)	PAN (20/24, 13 severe); onset ≤ 10 years in 18, ≤ 6 years in 14; oldest onset, cPAN at 59 years. 10/19 had visceral, 8/19 neurologic involvement, mainly peripheral	R169Q/R169Q R169Q/R169Q G47R/G47R	PAN predominant presentation of vasculopathy; variable age of onset and severity. Anti-TNF effective in 10/13 with severe visceral PAN
[13]	1	Turkish child with infl. strokes	G47R/R306*	Sib 1, successful HSCT for RCA.
[14•]	2 (1)	Sib 1: RCA, neutropenia, infl, HSM, hypercoagulable. Sib 2: profound lymphopenia, hypogam, infl, HSM; no vasculopathy	R169Q/R169Q	Sib 2: stable on anti-TNF but persisting lymphopenia, infl
[15•, 16]	2 (1)	CID (defective T cell proliferation), autoimmune cytopenias, lymphoproliferation; no overt vasculitis, but elevated serum IL-6	R169Q/R169Q	Sib 1 required HSCT (successful); sib 2 responded to sirolimus
[17•]	1	HHV8-negative Castleman disease (infl, HSM, generalized lymphadenopathy, polyclonal hypergam, anemia, thrombocytosis. Increased IL-6 (serum, lymph node biopsy)	G47R/G47R	Tocilizumab effective
[18•]	5 (1)	5 Portuguese sibs with Sheddon syndrome (onset of livedo in the 2nd decade, strokes in 4/5)	T119A/G142S	Adult onset of livedo and stroke
[19••]	2 (2)	Unrelated (French, Pakistani). PAN with neurologic involvement (central and peripheral), cPAN, PAN (severe visceral)	R169Q/P193L G47R/G47R	Peripheral blood IFN signature; genome-wide overexpression of neutrophil genes. Propose ADA2 as regulator of neutrophil activation
[20, 21]	2 (1)	cPAN; sib 1: immunodeficiency with low IgA, HSM, portal hypertension	K55*/N370K	
[22]	2 (1)	Dutch sibs, infl, HSM, early-onset strokes, and ophthalmologic and other cranial nerve involvement	R169Q/R169Q	
[23•]	6 (5)	Turkish; early-onset PAN (3 were reported in [2••])	G47R/ G47R	FFP of no or transient benefit. 1 patient had amyloidosis with renal involvement
[24]	2 (1)	Sri Lankan sibs; neutropenia, mucocutaneous infections, cPAN. Sib 1 died at 16 years old with septic shock; sib 2 now on anti-TNF	770-kb del/770-kb del	Deficient in both IL-17 receptor A (IL-17RA) and ADA2. High circulating and tissue levels of IL-1 β , IL-6, and TNF- α during inflammatory episodes
[25]	1	Infl, e nodosum, polyarthralgias, cPAN, PAN (visceral)	F355L/E328K	Intra-familial heterogeneity;
[26]	15 (3)	10/15 symptomatic: cPAN (5), PAN (4), stroke (6), peripheral nerve (2), lymphopenia (6); hypogam (6). 5/15 asymptomatic (age 5–42 years)	P251L/P251L G47R/G47R P251L/c.114delC MIT/R169Q	9/10 symptomatic patients treated with anti-TNF agents
[27]	2 (1)	cPAN, PAN, myositis, stroke; ANA+ (1:800); lymphopenia; low IgM, IgA	Y453C/V325Tfs*7	IFN signature present; neutrophil genes not overexpressed. No cytokine elevation; good response to anti-TNF
[28•]	5 (4)		F178S/F178S L451F/L451F	In 2 cases, PRCA was the sole problem

Table 1 (continued)

Reference	No. of patients (families)	Phenotype	CECR1 mutations (no. of patients with genotype)	Comment
[29-]	9 (6)	PRCA(2); combs + hemolytic anemia (1); pancytopenia, fever, HSM, lymphadenopathy. 1 patient died, possibly immunodeficient. None of 5 patients had vasculitis Infl, cPAN, stroke in 6/9; HSM, cytopenias, CVID. Lymphopenia (mainly B cell) and neutropenia eventually seen in all 9, but at diagnosis in only 2/9	R49Afs*13/R49Afs*13 D261Pfs*2/D261Pfs*2 R169Q/R169Q	Wide range in severity despite same genotype; most had long asymptomatic periods; 3 had severe or progressive disease. 5/9 on Ig replacement; 3/9 had HSCT for PRCA, pancytopenia, autoimmune thrombocytopenia CVID as manifestation of DADA2
[30]	2 (1)	CVID with progressive antibody deficiency and infections, but without vasculitis	M243R/R169Q	
[31•]	1	Monocytopenia, <i>M. avium</i> complex infection diagnosed as CVID	Q265*/Q265*	DADA2 masquerading as GATA2 deficiency; successful HSCT
[32]	1	Acute/subacute spastic paraplegia, cranial nerve dysfunction, PAN; transient anti-PL antibody	G47R/Y453C	
[33]	1	Ischemic mesencephalic stroke at age 2 years without cerebral vasculitis, preceded systemic inflammatory disease	G47A/R169Q	Failed anti IL-6 but responded rapidly to anti-TNF therapy
[34•, 35•]	1	Refractory atypical PRCA, age 4 years; mild immunodeficiency	N370K/G358R	Marrow failure resolved after HSCT, with normalization of plasma ADA2 activity
[36]	15 (11)	Early-onset PAN, livedo reticularis, or stroke. None had cytopenias or serious hematological abnormality, or recurrent infections, but 3 received Ig supplementation.	R231*/E328D (2) T360A/T360A (5) L249P/P344L (2) G47V/? (2) G47A/P251L Y453C/Y453C c.138_144delG/T360A L249P/T360A	Tested for DADA2 in 48 patients (43 families) with PAN phenotype. Several patients improved on steroids, but relapsed with tapering; 9/10 patients treated with anti-TNF had complete clinical remission
[37••]	11* (9) *2 were sibs of DADA2 patients [30]	5/15 presented with Ig deficiency (CVID), 4 also had vasculopathy (2 had strokes). Age of onset 2–33 years (median 8 years); at evaluation 13–51 years (median 22 years); wide range of symptoms (livedo, e nodosum, infections (9/11), arthralgias, PAN, cPAN, myositis, sinusitis, aphthous stomatitis, meningitis); lymphopenia (5), neutropenia (2). Normal T cell compartment, but most had B cell abnormalities (low IgM, low IgG in 10/11); progressive decrease in B cell function	V458D/V458D C408Y/c.542+1G>A R169Q/M243R G47A/G47A R169Q/R169Q c.973-1G>A/c.973-1G>A c.972+3A>G/c.973-2A>G	Test for DADA2 in 181 patients (43 families) with antibody deficiency; 9/11 with DADA2 received Ig supplementation; 8 received immunosuppression, 3 anti-TNF (improved immune function in some)
[38]	2 (1)	Early-onset recurrent stroke, febrile episodes, arthritis, oral ulcers, discoid rash, livedo racemosa. High titer antinuclear antibody. Constitutive type I interferon signature in peripheral blood.	G47R/C408Y	Unusual for autoimmune phenotype; type I interferon activation

Abbreviations: DADA2, deficiency of ADA2; *Infl*, inflammation; *HSM*, hepatosplenomegaly; *hypogam*, hypogammaglobulinemia; *PAN*, polyarteritis nodosa; *cPAN*, cutaneous PAN; *CID*, combined immunodeficiency; *CVID*, common variable immunodeficiency; *HSCT*, hematopoietic stem cell transplantation; *RCA*, red cell aplasia; *PRCA*, pure RCA; *FPF*, fresh frozen plasma

inflammatory features of DADA2 in one of two affected siblings who both had markedly elevated serum IL-6 levels [15•]. A patient with Castelman-like disease and high serum IL-6 levels responded to tocilizumab [17•].

PRCA and other kinds of hematologic dysfunction have generally not responded well to immunosuppressive agents, such as azathioprine, MMF, and cyclosporine (there is little information about anti-TNF therapy in such patients). As bone marrow-derived monocytes and macrophages are the main source of secreted ADA2, it was postulated early on that hematopoietic stem cell transplantation (HSCT) might be of benefit, although there was concern that the inflammation and vasculopathy associated with DADA2 might increase the risk for complications in the peri-transplant period, such as stroke or sinusoidal obstruction syndrome (SOS). HSCT has been performed to date in more than a dozen patients with severe hematologic or immunologic involvement, and has been successful in all cases, in spite of various complications [14•, 15•, 35•, 40••]. Restoration of normal plasma ADA2 activity has occurred in all the cases, as early as 2 weeks post transplant, and all disease manifestations appear to have resolved, with a median follow-up of 18 months. In some patients transplanted prior to the discovery of DADA2, benefit has persisted with follow-up of 5–10 years.

As ADA2 is found in plasma, the possibility of treating its deficiency with infusions of fresh frozen plasma (FFP) has been considered. However, the half-life of ADA2 after administration of FFP appears to be too short to make this practical [39]. PEGylated bovine ADA1 has been used for more than 20 years as replacement therapy for patients with SCID due to ADA1 deficiency, and effective gene therapy for that disorder is now available. The possibility of developing analogous therapies for DADA2 is under investigation.

The Physiological Function(s) of ADA2

The function of ADA2 in humans and the pathogenesis of DADA2 are not understood, but are the subject of two main avenues of speculation. One hypothesis assumes that an important function of extracellular ADA2 must be to *degrade extracellular Ado* and thereby to control signaling through one or more of the four known G protein-coupled Ado receptors (A1, A2A, A2B, A3). Loss of ADA2 might be expected to result in elevated levels of Ado in plasma, possibly derived from excessive ATP breakdown at sites of inflammation or ischemia. The second hypothesis is that ADA2 is a *growth or differentiation factor* that acts by binding to unidentified cell-associated receptors to regulate the inflammatory process.

There is no evidence that loss of ADA2 alters the level of extracellular Ado. The concentrations of Ado and dAdo were normal ($< 0.4 \mu\text{M}$) in plasma of a patient with

DADA2 (and in several others we have studied) [15•]. By contrast, in spite of having normal plasma ADA2 activity, these levels approach or exceed $10 \mu\text{M}$ in patients with SCID due to ADA1 deficiency [41]. These findings are consistent with the substrate affinities of ADA1 and ADA2, and the role of nucleoside transport in Ado metabolism. Thus, human ADA2 has very low affinity for Ado (and dAdo), with a Michaelis constant (K_m) of $\sim 2 \text{ mM}$, $\sim 10,000$ -fold above the normal concentration of Ado in plasma and extracellular fluid ($\sim 0.2 \mu\text{M}$). Cells possess bidirectional nucleoside transporters, which have higher affinity for Ado than ADA2 and function to rapidly equilibrate intra- and extracellular Ado concentrations. The concentration of free Ado in cells is kept vanishingly low by the actions of intracellular ADA1, which is a very efficient enzyme with a K_m for Ado of $\sim 20 \mu\text{M}$, and Ado kinase ($K_m \sim 1 \mu\text{M}$). As a result, any Ado generated in the extracellular space can very rapidly be taken up by erythrocytes or other nearby cells, where it is efficiently metabolized. Even without invoking any effect of so-called ecto-ADA (extracellular ADA1 bound to the glycoprotein CD26 present on many epithelial cells), these findings all support the conclusion that intracellular ADA1 plays a much greater role than extracellular ADA2 in controlling extracellular Ado. This must be true in mice, which lack both ADA2 and CD26-bound ADA1 [42]. Nevertheless, if one still believes that elevated extracellular Ado plays a significant role in the pathogenesis of DADA2, it might be worth considering a trial of PEGylated ADA1, which works by degrading Ado and dAdo in plasma after parenteral administration and is approved for treating SCID due to ADA1 deficiency.

That ADA1 and ADA2 are not functionally redundant is evident from the very different metabolic and clinical consequences associated with their deficiencies. This makes the hypothesis that secreted ADA2 is a growth or differentiation factor-attractive, but it is useful to appreciate that this notion arose from a fundamental misinterpretation of experimental findings. It derives from the isolation 20 years ago of a secreted “Insect Derived Growth Factor (IDGF),” renamed “ADAGF” after it was found to be a very active ADA, with a K_m comparable to that of “mammalian cytoplasmic ADA,” i.e., ADA1 [43, 44]. The “growth stimulation” attributed to ADAGF was totally dependent on its ADA activity, and the phenomenon was shown more accurately to be a relief of growth inhibition caused by exogenous Ado, which was present at toxic concentrations in fly cell culture medium; the same apparent effect on cell growth could be achieved by adding bovine ADA1 [44, 45]. *CECR1* was identified as the human homolog of IDGF/ADAGF [46], and ADA2 was shown to be its product, albeit far less enzymatically active than invertebrate ADAGFs [7, 47].

The hypothesis that secreted human ADA2 is a growth or differentiation factor has not been investigated *in vivo*, as a suitable animal model is lacking. However, Zavialov and his colleagues have reported that both exogenously added human ADA2 and ADA1, *independent of enzymatic activity*, could promote the division of cultured human T cells, and that ADA2, but not ADA1, could stimulate the T cell-dependent differentiation of monocytes into macrophages. These *in vitro* findings were highly dependent on the concentrations of ADA1 and ADA2 employed, and on other experimental conditions [6]. Zavialov's laboratory has shown that ADA2 binds avidly to heparin sulfate and proteoglycans present on many cell types, although the consequences of this interaction are unknown [7]. More recently, these investigators have studied the ability of ADA2 to bind to various cells involved in immunity or inflammation [48]. A critical discussion of these experimental findings and their potential physiological relevance is beyond the scope of this review.

Conclusions

Almost 4 years after the first reports of its existence, DADA2 has entered the differential diagnosis of several serious conditions of interest to both pediatric and adult rheumatologists, including PAN and other forms of vasculitis, the autoinflammatory disorders, and disorders characterized by immune deficiency and dysregulation. In a subset of DADA2 patients, bone marrow failure dominates the clinical picture. Fortunately, many patients respond well to anti-TNF agents, which are employed by rheumatologists to treat RA and other diseases characterized by systemic inflammation. Because the manifestations of DADA2 are so varied, and the complications of vasculopathy potentially catastrophic, it behooves rheumatologists to appreciate the ways in which DADA2 may present, and to have a high index of suspicion, particularly in patients with compatible features who lack typical biomarkers for other conditions under consideration, or do not respond satisfactorily to immunosuppressive therapies. The diagnosis of DADA2 can be made or excluded not only by *CECR1* gene sequencing but also by a relatively straightforward enzyme assay.

Compliance with Ethical Standards

Conflict of Interest Michael S Hershfield receives grant support from Leadiant Biosciences, Inc., the manufacturer of Adagen, a PEGylated adenosine deaminase used to treat patients with ADA1 deficiency.

Hasan Hashem, Susan J Kelly, and Nancy J Ganson declare they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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