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Review

Antipurinergic therapy for autism—An in-depth review

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ABSTRACT

Are the symptoms of autism caused by a treatable metabolic syndrome that traces to the abnormal persistence of a normal, alternative functional state of mitochondria? A small clinical trial published in 2017 suggests this is possible. Based on a new unifying theory of pathogenesis for autism called the cell danger response (CDR) hypothesis, this study of 10 boys, ages 5–14 years, showed that all 5 boys who received antipurinergic therapy (APT) with a single intravenous dose of suramin experienced improvements in all the core symptoms of autism that lasted for 5–8 weeks. Language, social interaction, restricted interests, and repetitive movements all improved. Two children who were non-verbal spoke their first sentences. None of these improvements were observed in the placebo group. Larger and longer studies are needed to confirm this promising discovery. This review introduces the concept of M2 (anti-inflammatory) and M1 (pro-inflammatory) mitochondria that are polarized along a functional continuum according to cell stress. The pathophysiology of the CDR, the complementary functions of M1 and M2 mitochondria, relevant gene-environment interactions, and the metabolic underpinnings of behavior are discussed as foundation stones for understanding the improvements in ASD behaviors produced by antipurinergic therapy in this small clinical trial.

1. Background

In over 20 years of modern clinical trial efforts (McPheeters et al., 2011) and 75 years since the first description of autism (Kanner, 1943), no drug has been FDA approved to treat the core symptoms of autism spectrum disorder (ASD). I believe this is because the root cause of ASD is not yet understood. This has made it impossible to develop a unifying theory of pathogenesis that might help to guide new drug development.

2. The cell danger response hypothesis

The Suramin Autism Treatment 1 (SAT1) study (Naviaux et al., 2017) was the first clinical trial to test a new unifying theory for the root cause and a new treatment of autism. The cell danger response hypothesis represents a paradigm shift in how scientists think about the cause of autism. Instead of focusing on a particular behavior, cell type, genes, the microbiome, synapses, or the connectivity of neural circuits in the brain, the cell danger hypothesis states that the root cause of autism is a universal cellular response to stress that shifts normal cell function to a new state. Severe and/or prolonged stress forces a reallocation of cellular resources for survival. This universal response to stress traces to mitochondria and is called the cell danger response (CDR) (Naviaux, 2014). Aspects of the CDR are also referred to as the integrated stress response (Green et al., 2011; Nikkanen et al., 2016; Silva et al., 2009). The CDR gives the appearance of mitochondrial dysfunction, but is actually a normal, necessary, and *highly regulated change* in mitochondrial function from oxidative phosphorylation to cellular defense. This shift is needed to respond to a threat, and to heal after an injury. Mitochondria that defend the cell in danger can no longer function the same as they do under unstressed conditions (Naviaux et al., 2009). This programmed change in mitochondrial function is needed for innate immunity and inflammation (West, 2017), which in turn are required for establishing the adaptive immune response and healing.

3. M1 and M2 mitochondria

To emphasize the importance and dynamic nature of this programmed change in mitochondrial function, I have designated these as M1 and M2 mitochondria (Fig. 1). M1 and M2 mitochondria represent two poles on a functional continuum (Sander and Garaude, 2017) regulated by the CDR. M2 mitochondria are devoted to oxphos and are anti-inflammatory. In contrast, M1 mitochondria are pro-inflammatory. M1 mitochondria are specialized for creating the oxidative shielding response (Naviaux, 2012b). M1 mitochondria are tasked for cellular defense and increase cellular redox (are pro-oxidants) and perform dozens of other functions needed for anti-viral and anti-microbial defense. The shift from M2 to M1 mitochondrial functions is an intrinsic feature of an activated CDR. Within a given cell, this shift creates a spectrum from 100% M2 mitochondria when stress is minimum, to

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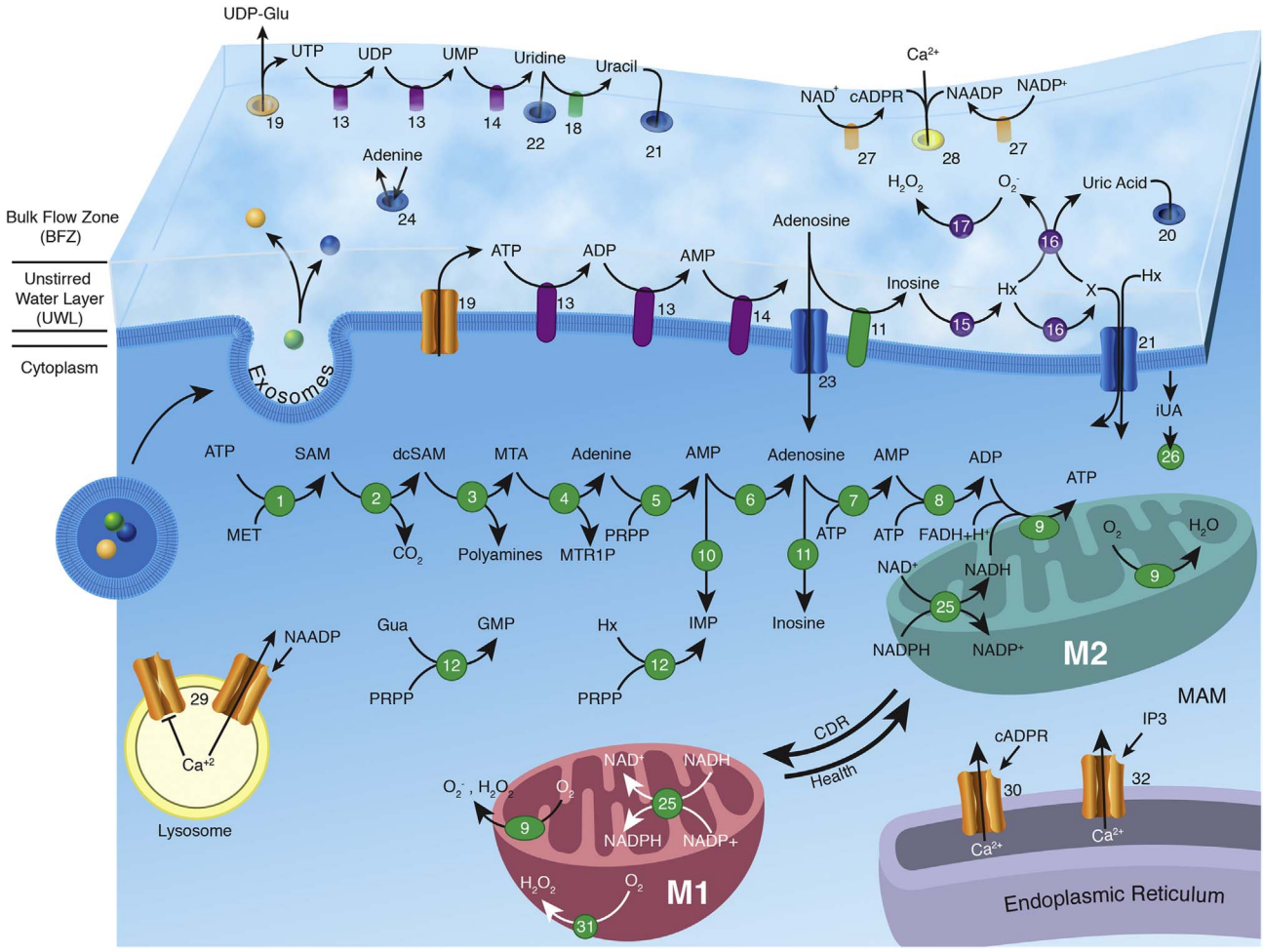

Fig. 1. Metabolic control of purinergic signaling. The availability of purinergic effectors in the unstirred water layer where receptors and ligands meet is controlled by a suite of metabolic proteins and solute channels. A simplified summary of their actions, and the dynamic capacity for mitochondrial functional changes that are associated with the CDR are illustrated.
Cellular zones: Bulk flow zone (BFZ), unstirred water layer (UWL), cell membrane, cytoplasm, ER-mitochondria-associated membranes (MAMs).
Organelles: M1 mitochondria are pro-inflammatory and dedicated to helping to orchestrate the cell danger response (CDR). M2 mitochondria are anti-inflammatory and dedicated to oxidative phosphorylation (oxphos). Lysosomes contain an acidic pool of calcium gated by NAADP acting on TCP. Endoplasmic reticulum (ER) contains a neutral pool of calcium gated by cADPR acting on the ryanodine receptor (RyR). Exosomes are sphingolipid-enriched nanovesicles that are used for cell-cell signaling and the removal and exchange of intracellular materials.
Proteins: Integral Membrane-associated enzymes (purple), Non-Integral membrane enzymes (green), Receptors and Ligand-gated channels (orange), and Non-ligand-gated Transport Channels (blue). 1—MAT: methionine adenosyl transferase. 2—SAMdc: S-Adenosylmethionine (SAM) decarboxylase. 3—dcSAM aminopropyltransferases; spermidine synthase and spermine synthase. 4—MTAP: methylthioadenosine phosphorylase. 5—APRT: adenine phosphoribosyl transferase. 6—5NT: 5′-nucleotidase. 7—ADOK: adenosine kinase. 8—ADK; adenylate kinase (also known as myokinase). 9—The mitochondrial electron transport chain (ETC), consisting of mitochondrial respiratory chain complexes I, II, III, IV, and V. 10—AMPD2: AMP deaminase 2. 11—ADA: adenosine deaminase. 12—HGPRT: hypoxanthine-guanine phosphoribosyl transferase. 13—CD39: ectonucleoside triphosphate diphosphohydrolase (also called ENTPD1), 14—CD73: ecto-5′-nucleotidase (also called NT5E). 15—PNP: purine nucleoside phosphorylase. 16—XO: xanthine oxidase. 17—SOD3: copper-zinc dependent ecto-superoxide dismutase 3. 18—Upase: uridine phosphorylase. 19—Pannexin/P2X7 Porin; activated by oxidation and ATP, and blocked by suramin. 20—GLUT9a/SLC2A9: cytokine regulated uric acid transporter. 21—SNBT1/SLC23A4: sodium-dependent, concentrative nucleobase transporter 1. 22—ENTI/SLC29A1: equilibrative nucleoside transporter 1. 23—ENT2/SLC29A2: equilibrative nucleoside transporter 2. 24—ENBT1/SLC43A3: equilibrative nucleobase transporter 1. 25—NNT: nicotinamide-nucleotide transhydrogenase. 26—NLRP3 inflammasome assembly. 27—CD38: cyclic ADP ribose hydrolase. 28—TRPM2: transient receptor potential cation channel, subfamily M, member 2. 29—TPC: two pore channel. 30—RyR: ryanodine receptor. 31—NOX4: NADPH oxidase 4. 32—IP3R: inositol trisphosphate receptor.
Metabolites: Met: methionine. ATP: Adensoine triphosphate. ADP: adenosine diphosphate. AMP: adenosine monophosphate (also called adenylate or adenylic acid). SAM: S-adenosyl methionine. dcSAM: decarboxyl-SAM. Polyamines: spermidine, spermine. MTA: methylthioadenosine. MTR1P: methylthioribose-1-phosphate. PRPP: phosphoribosylpyrophosphate. Hx: hypoxanthine. Gua: guanine. X: xanthine. eUA: extracellular uric acid. iUA: intracellular uric acid. UTP: uridine triphosphate. UDP: uridine disphosphate. UMP: uridine monophosphate (also called uridylate or uridylic acid). UDP-Glu: UDP-glucose. NADH: nicotinamide adenine dinucleotide (reduced form). NADPH: Nicotinamide adenine dinucleotide phosphate (reduced form). cADPR: cyclic ADP ribose. NAADP: nicotinic acid adenine dinucleotide phosphate. IP3: inositol trisphosphate.

50:50 M1:M2 and intermediate forms at medium-stress conditions, up to 100% M1 when the survival of the cell is threatened. When the fusion-fission cycle of mitochondria in a particular cell type is rapid (minutes to hours), as occurs in rapidly dividing cells in the bone marrow and gut epithelium, this transition comes to a new steady-state within minutes to hours. When the fusion-fission cycle of mitochondria is slow (for example, it is 2 weeks in the cardiac myocytes of adult mice (Song and Dorn II, 2015)), the M2 to M1 transition occurs more slowly, and the M1 state lasts longer after the danger has passed. Other differentiation states of mitochondria exist. For example, the mitochondria in brown adipose tissue (BAT) and beige/bright cells in white adipose tissue (WAT) have distinct developmental origins (Kajimura and Saito, 2014). Pluripotential M0 mitochondria are present in stem cells (Folmes et al., 2012) and primary oocytes (Van Blerkom, 2011).

The shift from M2 (anti-inflammatory) to M1 (pro-inflammatory) mitochondria that occurs when a cell becomes threatened is not unlike the analogous shift in the functional state of macrophages from resting

M0 or polarized M2, to M1. The M1 phenotype of macrophages is pro-inflammatory and needed for cell defense, while M2 macrophages are anti-inflammatory and needed to facilitate the resolution of inflammation and healing. This cellular polarization is strongly correlated with oxidative phenotype of the mitochondria within macrophages (Chen et al., 2017). When the choreographed sequence of metabolic steps in the healing cycle encounters a roadblock for any reason, a persistent form of the CDR results, and M1 pro-inflammatory mitochondria persist past the time they are needed. This changes the trajectory of child development, and can lead to ASD and several other disorders.

4. ASD genetics, the CDR, and inflammation

Each of the common genes known to strongly increase the risk of ASD can be shown to play a role in CDR signaling or maintenance. Fragile X is just one of several examples. The Fragile X gene FMR1 encodes a protein that normally inhibits the translation of proinflammatory cytokines like TNFα (Garnon et al., 2005). Decreased expression of the Fragile X protein results in persistent activation of the CDR in the form of a low-grade inflammatory response in the brain (Di Marco et al., 2016). Could therapy directed at the CDR be effective in Fragile X, and other genetic causes of ASD? Antipurinergic therapy (APT) directed at the CDR has already proven effective in correcting ASD-like behaviors in the Fragile X mouse model (Naviaux et al., 2015).

Rett syndrome is another example of a genetic cause of autism spectrum disorder that is tied to the cell danger response and inflammation (Cortelazzo et al., 2014). Most cases of Rett syndrome trace to new mutations in the MeCP2 gene, which codes for a methyl-CpG binding protein. Mutations in MeCP2 alter chromatin structure and lead to retroelement mobilization (Muotri et al., 2010), genetic instability, and profound changes in the innate immune response (Derecki et al., 2012). The CDR coordinates retroelement mobilization and innate immunity (Naviaux, 2014). Animal models of Rett syndrome are an important reminder that even in some genetic causes of autism, the core behavioral features of ASD are not permanent. Behaviors can be reversed by treating the cell danger response that underlies oxidative changes and inflammation (Derecki et al., 2013).

Other examples include Angelman and Smith-Magenis syndrome. Angelman syndrome is usually caused by a *de novo* maternal deletion or mutation of a gene called ubiquitin-protein ligase E3A (UBE3A) located on chromosome15q11-q13. UBE3A is involved in the unfolded protein response. Mutations in UBE3A result in the accumulation of unfolded proteins (Mishra et al., 2009), which are in turn known to activate the CDR (Smith, 2014). Smith-Magenis syndrome is usually caused by chromosomal copy number variation (CNV) resulting in a deletion of a patch of DNA on chromosome 17p11.2 containing the retinoic acid induced 1 (RAI1) gene (Huang et al., 2016). This is not to be confused with the retinoic acid induced gene 1 (RIG1), a helicase on chromosome 9. Defects in RAI1 result in increased childhood infections and immunologic abnormalities (Perkins et al., 2017) that result in repeated activation of the CDR. Future studies will be required to test therapies directed at the CDR in children with Fragile X, Rett, Angelman, and Smith-Magenis syndromes.

5. Cellular order, metabolism, defense, and immunity

5.1. Mitochondria and the CDR

The daily operations of the cell require protein-protein and many other macromolecular interactions that rely critically on cellular spatial order—packing—for efficient function. When order is disrupted, function suffers. Any kind of physical or biological injury to the cell decreases electrons shuttled from nutrients to mitochondria. Mitochondrial electron flow acts as a barometer of cellular health. When mitochondrial electron transfer is disrupted a cellular metabolic syndrome is produced. This new cellular metabolic state is needed for healing. This starts locally at the site of injury, but propagates to neighboring cells as they adopt a change in function to contain the injury. If the injury cannot be contained locally, systemic signals are sent by neuroendocrine and autonomic nervous systems that ultimately produce changes in systemic metabolism and behavior. Disruptions in molecular order or the organization of cytoskeleton and organelles within cells is perhaps one of the most fundamental signals of danger. Increasing cellular disorder is the biologic equivalent of increasing thermodynamic entropy.

5.2. The importance of water

The decrease in the ordered state of macromolecules and solutes forces a change in the distribution, behavior, and thermodynamic properties of water molecules (H_2O) in and around cells (Chaplin, 2006; Pollack, 2013; Prigogine and Nicolis, 1971). The partial positive and negative charge on a molecule of water, and other forces, constrain its movement by interacting with the surface charges around proteins, membranes, and cytoskeleton creating a fraction of bound or “vicinal” water that covers all biological surfaces. The more polymers or membranes in solution, the greater the fraction of bound to unbound water. The net effect of increased acidity and increased dissolved oxygen in cells and tissues is to inhibit macromolecular (polymer) synthesis reactions. Polymer synthesis reactions include the condensation of amino acids to make proteins and nucleotides to make RNA and DNA. This decreases the differentiated functional capacity of the cell, but is required to initiate healing after injury. Mitochondrial fusion-fission dynamics shift toward fission to permit increased quality control under stress (Youle and van der Bliek, 2012). Metabolic synapses between mitochondria and the endoplasmic reticulum known as mitochondrial-associated membranes (MAMs) also change under stress, further altering the ordered intracellular structure of organellar networks. MAMs regulate calcium, phospholipid, sphingolipid exchange, and many other key physiologic processes (Sano et al., 2009). Changes in mitochondrial dynamics during cell stress in tissues link increasing cytoplasmic disorder with increasing disorder of water molecules, and an increase in CDR-associated functions. The monitoring of cellular disorder is fundamental for normal immune system function (Cunliffe, 1997).

6. The CDR, redox, M1 mitochondria, and ASD

Oxidative changes in autism have been well-studied (James et al., 2006; Rose et al., 2017). Single cells make reactive oxygen species (ROS) to harden themselves when they come under attack by pathogenic organisms or environmental stress (Naviaux, 2012b). Indeed, the mitochondria in cells make the very ROS that inhibit their own bioenergetic functions. This seems self-destructive, but it is not. The adaptive function of the ROS-response cannot be understood within a single-cell frame of reference. The relevant frame of reference is the host considered as a collective system of many cells and tissues. Local ROS are responsible for activating a normal, but latent, alternative function of mitochondria in threatened cells. Under baseline conditions, tissue mitochondria exist mostly in an M2, or anti-inflammatory state dedicated to oxphos. When ROS are increased, mitochondria take on a new job. They become polarized to M1 mitochondria, become an important source of ROS themselves, increase the rate of damaged organelle removal via intracellular quality control methods, and become the initiators and coordinators of the antiviral response (Seth et al., 2005) and cellular defense (West et al., 2011). To do this, mitochondria must temporarily drop their “day job” as the hub of oxidative phosphorylation served by their M2 polarized state. The cell-autonomous inhibition of mitochondrial oxphos by ROS decreases both the production of cellular building blocks and the exchange of building blocks with other cells. The ROS-response and M1 polarization of mitochondria is adaptive because it decreases the chances that a virus, or pathogenic

organism, or damage from a traumatic or chemical injury or toxin can spread to kill the host. A line of cells in which mitochondrial oxphos is decreased, and lactic acid and ROS are increased, creates the cellular equivalent of sealing the bulkheads that separate the compartments on a damaged ship or submarine to prevent the spread of more water to undamaged sections. Furthermore, when the metabolic rate of a single cell is decreased relative to neighboring cells, the local clock of biological time within that cell slows, permitting it to resist maturation and outlast the cells unable to use fewer resources for survival. For this and other reasons, the author favors the term "oxidative shielding" instead of "oxidative stress" (Naviaux, 2012b). The host benefits strongly from the ability of single cells to shift nimbly from peacetime M2 metabolism to defensive M1 metabolism needed for damage containment in response to environmental stress. When viewed contextually, it can be understood that this is not "mitochondrial dysfunction". The M1 state is an adaptive, new function of mitochondria that is produced when cells come under stress. Chronic disease results when this cell danger response cannot be turned off when its job is done.

7. The CDR, exosomes, and the immune response in ASD

Stressed cells increase the number and diversity of lipid nanovesicles called exosomes that they release into the extracellular space (Fig. 1) (Nemeth et al., 2017). Exosomes are enriched in stress- and danger-signaling sphingolipids, self-antigens that include metabolic enzymes from internal compartments of the cell, micro-RNAs, mitochondrial DNA (mtDNA), and partially processed materials from the mitophagy and autophagy pathways (Pellegrino and Haynes, 2015). Exosomes help to remove non-infectious aggregates of proteins like β-amyloid, α-synuclein, and tau that might otherwise accumulate inside the cell and become toxic (Rajendran et al., 2006; Wang et al., 2017; Yang et al., 2017). Exosomes are also critical for many forms of cell-cell communication involved in fundamental biological processes like fertilization (Machtinger et al., 2016) and neurotransmission (Lachenal et al., 2011). Viruses and microbial pathogens are known to hijack exosomes for cell-to-cell transmission. To combat this, certain interferon-induced genes like ISG15 have evolved that decrease total cell protein synthesis and exosome release from infected cells. This creates a reduction in cell-to-cell communication through exosome release, but permits more efficient disposal of pathogens within the cell by fusion with lysosomes (Villarroya-Beltri et al., 2017).

Another function of exosomes is to permit the export and recycling of biochemical building blocks produced during normal cell function (Fig. 1). Exosome release and reuptake into neighboring or distant cells occurs naturally during the turnover of billions of cells that occurs daily by apoptosis. Naturally occurring molecules like mtDNA, α-galactosylceramide in lipid rafts, formyl-methionine containing mitochondrial peptides, released ATP and UTP, and other molecules can stimulate toll-like receptors (TLRs) and related stress-sensing receptors on B1 cells and support the production of naturally occurring, low-affinity, IgM antibodies. These natural autoantibodies (NAAs) are present from birth, are produced by T-cell independent, TLR-stimulated B1 cells, and have potent anti-inflammatory effects (Lobo, 2017). Low-titer IgM antibodies to self-antigens are increased after surgery and other traumas (Raad et al., 2014) because of increased release of material from stressed or damaged cells (Oka et al., 2012). These natural autoantibodies play an important protective role, limiting inflammation (Andaluz-Ojeda et al., 2013). Once the trauma or cellular damage is healed, exosome production, and other cellular sources of antigens are diminished, and autoantibody production is returned to background levels. If antigen presentation continues because of ongoing stress or infection, then high-affinity IgG antibodies can be stimulated, selected, and amplified.

Autoantibodies to the folate receptor (Frye et al., 2016a), and maternal antibodies to brain proteins (Edmiston et al., 2017) are associated with ASD risk. Complex neuroimmune syndromes like pediatric acute-onset neuropsychiatric syndrome (PANS), pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (PANDAS), and autonomic disturbances like postural orthostatic tachycardia syndrome (POTS) are also risks in ASD. The chronically activated CDR can also lead to sleep disturbances, seizures, leaky gut, gut inflammation, and dysmotility. Thyroid abnormalities reflected by an increase in the rT3 also occur (Frye et al., 2017). This may be a consequence of stress induction of the selenoproteins, deiodinases 2 and 3 (DIO2 and 3), that are responsible for inactivating T4 and producing reverse T3 (rT3) (Lamirand et al., 2008). The CDR also regulates Th17 cells through purinergic signaling (Sullivan et al., 2014). Th17 cells and Th17 receptor expression on monocytes play several important roles in regulating breaches in immune tolerance (Pfeifle et al., 2017), and inflammation in autism (Nadeem et al., 2017).

8. The CDR, natural infections, and vaccinations

The CDR is a normal and universal feature of any stress. This means that it is normally activated by both natural infections and vaccination. The CDR is needed to establish cellular and humoral immunity. Since the large majority of children and adults who receive vaccinations, or are exposed to common natural viral infections like Epstein Barr Virus (EBV), are able to recover without incident, the biological question is, "Why are some children and adults unable to moderate or turn off the CDR when its job is done and immunity is established?" We do not have an answer to this question yet. However, relatively simple measures like distributing vaccinations over time, instead of giving a large number at once would decrease the chances of triggering an excessive CDR in individual children deemed to be at increased risk. In addition, future research into the metabolomic phenotypes of children before and after vaccinations may begin to shine some light on differences in natural metabolic and physiologic states, that might permit us to predict the risk and develop treatments to prevent the rare complications like post-immunization, febrile seizures (MacDonald et al., 2014).

9. Gene-environment interactions in ASD

9.1. Managing environmental risks

The CDR is triggered by both genes and environmental factors. Genes, and the proteins they make, can be thought of as providing adaptive resilience, like stretch in a homeostatic safety net, to a large number of stressors. When new, more frequent, or more severe exposures are encountered, the adaptive and healing capacity of cells can be pushed to, and beyond its homeostatic limits. This creates roadblocks to healing that result in a persistent form of the CDR. Environmental factors that can test the resilience of any given genotype include physical, chemical, nutritional, microbial, and psychological traumas. Infections during pregnancy (Zerbo et al., 2013), prenatal maternal psychological stress (Kinney et al., 2008; Ronald et al., 2010), early life stress (Cameron et al., 2017; Heun-Johnson and Levitt, 2016; Rutter et al., 2001), and exposure to a variety of toxins (Braun et al., 2014), metals (Kalkbrenner et al., 2014; Palmer et al., 2009), or traffic-related air pollution (Volk et al., 2011; Volk et al., 2013) have each been shown to contribute to ASD risk. Combinations of all these factors in a particular home, neighborhood, city, or rural environment contribute to the concept of total toxic load (Herbert et al., 2013). If the exposure happens during critical periods in early child development, ASD and several other childhood disorders can result (Landrigan et al., 2012). When avoidable risks are managed, pregnancy outcomes and child health can be improved (Adams et al., 2016; Schmidt et al., 2011).

9.2. Ecogenetics, ecoalleles, and ASD

Recent twin studies show that environmental factors are responsible for about 60% (mean = 58%; 95% CI = 30–80%) of ASD. The collective contribution of all genes was about 40% (mean = 37%; 95% CI

8–84%) (Hallmayer et al., 2011), but no single gene accounts for more than 1–2% of all of ASD (Talkowski et al., 2014). The term ecogenetics describes the interaction between genes and environment. Many genes show strong differences in function depending on exposure to different environmental factors. These "ecoalleles" are common gene variants—polymorphisms with allelic frequencies of about 2%–50%—in enzymes, receptors, transporters, and transcription factors that have different activities depending on environmental factors. Some of these environmental factors include seasonal and diurnal temperature fluctuations, or the availability of calories, fats or carbohydrates, trace metals, redox, critical cofactors like thiamine (B1), niacin (B3), riboflavin (B2), folic acid (B9), B12, lipoic acid, tetrahydrobiopterin (BH4), biotin, pantothenic acid, vitamin D, C, or pyridoxine (B6), or exposure to drugs, pesticides, or toxins.

The prevalence of ecoalleles in different populations around the world differs significantly according to different climatic, dietary, infectious disease, and cultural conditions. For example, the ecoallele c.677T in methylene tetrahydrofolate reductase (MTHFR) is rare in populations from sub-Saharan Africa (5%), but more common in Mexican, Italian, and Ashkenazi Jewish populations where it has an allelic frequency of about 50% (Karban et al., 2016). The risk of disease associated with the c.677T ecoallele is context dependent. Despite being more common in Ashkenazi Jewish populations, the c.677T allele in MTHFR was a risk factor for autism, inflammatory bowel disease, and certain other diseases in non-Ashkenazi populations but not in Ashkenazi populations (Frye and James, 2014; Karban et al., 2016). If ecoalleles were always harmful in all world contexts at all ages, they would eventually be removed from the ancestral gene pool, or reduced to frequencies well below 1%. Alleles that cause disease in post-reproductive adults can be maintained by advantages to children or young adults. In the case of MTHFR, it has been hypothesized that the c.677T variant confers resistance to malaria (Meadows et al., 2014). In sub-Saharan Africa, the sickle-cell hemoglobin trait became the most common genetic form of resistance to malaria. In other regions where different ecological and nutritional factors played a role in the selection of mechanisms of pathogen resistance that were not limited to malaria, MTHFR variants became more widespread, as in historical populations in southern Europe and Mexico.

Other ecoalleles include variants of cystathionine beta synthase (CBS), catechol-O-methyl transferase (COMT), monoamine oxidase A (MAO-A), amine oxidase, copper-containing 1 (AOC1; also known as diamine oxidase, DAO, and amiloride binding protein, ABP), histamine N-methyl transferase (HNMT), N-acetyltransferase 2 (NAT2), sulfotransferase 1A1 (SULT1A1), glucose-6-phosphate dehydrogenase (G6PD), extracellular super oxide dismutase 3 (SOD3), deiodinase 2 (DIO2), chitinase 1 (CHIT1), solute carrier 19A1 (SLC19A1, also called the reduced folate carrier 1, RFC1), methionine adenosyltransferase 1 (MAT1), sphingomyelinase phosphodiesterase 1 (SMPD1, also called acid sphingomyelinase, ASM), endothelial nitric oxide synthase (eNOS, also called NOS3), hemochromatosis (HFE), glutathione peroxidase 1 (GPX1), glutathione-S-transferase pi-1 (GSTP1), and serum paraoxonase/arylesterase 1 (PON1). Evolutionary selection maintains the prevalence of ecoalleles at frequencies of about 2%-50% because they have environment- and context-dependent fitness advantages, sometimes manifested only as the heterozygote carrier genotype.

9.3. The CDR activates the moonlighting functions of ecoalleles

As discussed above, M1 mitochondria use ROS to activate their latent/moonlighting function as coordinators of the cell danger response under conditions of environmental stress. Many metabolic enzymes also have moonlighting functions that are induced by new environmental conditions (Sriram et al., 2005). These moonlighting functions often seem self-destructive unless understood in terms of the cell danger response (CDR) (Naviaux, 2014). One important example is the mitochondrial enzyme dihydrolipoamide dehydrogenase (DLD), also known as the E3 protein. This protein is shared by 5 different mitochondrial enzyme systems that are critical for regulating cellular bioenergetics, redox, and amino acid metabolism. The enzyme systems catalyze NAD^+ dependent, oxidative decarboxylation reactions. They are: pyruvate dehydrogenase complex (PDH), alpha-keto glutarate dehydrogenase (AKDH, also known as 2-OGDH), branched chain ketoacid dehydrogenase complex (BCKDH), 2-oxoadipate dehydrogenase (2-OADH), and the glycine cleavage system. Each of these 5 key enzyme systems can be affected when DLD changes from its canonical function to its moonlighting function under conditions of environmental stress. This leads to CDR-associated chromatin remodeling because alpha ketoglutarate released from mitochondria is an essential cofactor for Jumonji histone demethylases (Kang et al., 2017), and NAD^+ build-up activates histone deacetylation and DNA transcriptional silencing by sirtuins (Simoneau et al., 2016). The net result is to slow gene expression and metabolism in stressed cells. Over 90 DNA variants of DLD are currently listed in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar?term=238331 [MIM]). Thirty-one of 92 variants (34%) are currently classified as benign or likely benign. Some of these may in fact be ecoalleles of DLD that have not yet been fully characterized. Several DLD variants are strongly inhibited by valproate, creating an ecogenetic risk for drug-induced liver toxicity (Kudin et al., 2017).

Under conditions of low mitochondrial matrix pH produced by ischemia-reperfusion, high salt concentration, and other stresses, DLD changes from its normal dimeric conformation and adopts an oligomeric or monomeric structure. The normal DLD activity is lost as the moonlighting functions of the E3 protein are activated by stress. The first new activity to emerge is reactive oxygen species (ROS) production (Ambrus and Adam-Vizi, 2017) using NADH and oxygen for oxidative shielding in response to microbial infection or other stress (Naviaux, 2012b). This accompanies the shift from M2 anti-inflammatory to M1 pro-inflammatory mitochondria. Progressive stress leads to the transformation to monomer configuration and unmasks a cryptic protease activity. The emergent E3 protease cleaves several mitochondrial substrates, including the iron-sulfur cluster biogenesis protein frataxin (Babady et al., 2007). This leads to a prolonged shift from M2 to M1 mitochondria after transient but severe stress (Klyachko et al., 2005). Disruptions in iron-sulfur cluster assembly can have profound affects on dozens of proteins in key pathways of the cell danger response. Among these are viperin and ABCE1 used in the antiviral response, aconitase in the Krebs cycle, subunits of the mitochondrial respiratory chain complexes I, II, and III needed for energy production, and DNA repair proteins like XPD and FANCJ (Braymer and Lill, 2017).

9.4. Emergent phenotypes, epigenetics, and metabolic treatments

Mixtures of ecoalleles, with and without moonlighting functions, produce new phenotypes and patterns of disease risk and resilience that represent latent traits that are revealed by exposure to specific environmental triggers. While single ecoalleles can have predictable consequences like common pharmacogenomic variants (Schuck and Grillo, 2016), mixtures of ecoalleles are conditional and have emergent phenotypes that cannot be predicted from genomic analysis alone. Ecoalleles create resilience in the homeostatic safety net that helps life manage environmental infections, toxins, famine, vascular and tissue injury, and other stressors. The combined effect of unique mixtures of ecoalleles is to create metabolic phenotypes that are key targets for natural selection and evolution. Emergent metabolic traits are the result of real-time interaction of genes and environment. Their fitness depends on the environmental context. What permits survival under harsh conditions may slow reproduction or development under mild conditions.

When environmental conditions change over the course of child development, and harsh conditions alternate with mild, or harsh conditions begin to be more common than mild, recovery from the survival or defensive cellular state can be delayed or persist. Some of the

Table 1
Metabolic disturbances in autism spectrum disorder.

No.	Metabolic abnormality	Authors	Dates	References
1	Decreased tryptophan conversion to serotonin; increased kynurenine pathway	H. Eldon Sutton	1958	Sutton and Read (1958)
2	Increased tryptophan and platelet serotonin	Daniel Freedman	1961	Mulder et al. (2004); Schain and Freedman (1961)
3	Increased purine metabolism	William Nyhan, Mary Coleman	1969, 2000	Nyhan et al. (1969); Page and Coleman (2000)
4	Pyridoxine metabolism	Bernard Rimland James Adams	1978, 2006	Adams et al. (2006); Rimland et al. (1978)
5	Increased sphingolipids and gangliosides	Chris Gillberg	1998	Nordin et al. (1998); Schengrund et al. (2012)
6	Decreased sulfation, and plasma sulfate; Increased plasma cysteine, urine sulfate	Rosemary Waring	1999	Alberti et al. (1999)
7	Mitochondrial DNA mutations	William Graf, Robert Naviaux, Richard Haas	2000	Graf et al. (2000)
8	Mitochondrial respiratory chain complex overactivity	William Graf et al. Luigi Palmieri and Tony Persico Richard Frye and Robert Naviaux Shannon Rose, Richard Frye,Jill James	2000 2010 2011 2014	Frye and Naviaux (2011); Graf et al. (2000); Palmieri et al. (2010); Rose et al. (2014a); Rose et al. (2014b)
9	Decreased cholesterol/sterols	Elaine Tierney, Richard Kelley	2000	Tierney et al. (2000)
10	Microbiome dysbiosis	Richard Sandler, Sydney Finegold	2000, 2002	Finegold et al. (2002); Sandler et al. (2000)
11	1-Carbon and folate metabolism	Jill James	2004	James et al. (2004)
12	Cysteine, glutathione, SAM/SAH	Jill James	2006	James et al. (2006)
13	Reactive oxygen metabolism	Jill James	2006	Frustaci et al. (2012); James et al. (2006)
14	Pyrimidines—increased uridine, BAIB	W. Brussel, James Adams	2006, 2011	Adams et al. (2011)Brussel et al. (2006); Micheli et al. (2011)
15	Creatine deficiency	V. Leuzzi, Sylvia Stoeckler-Ipsiroglu	2002, 2006	Leuzzi (2002); Mercimek-Mahmutoglu et al. (2006)
16	Mitochondrial control of epigenetics	Robert Naviaux, Keshav Singh, Doug Wallace	2008, 2010	Naviaux (2008); Smiraglia et al. (2008); Wallace and Fan (2010)
17	Vitamin D insufficiency	John Cannell, Chris Gillberg	2008, 2012	Cannell (2008); Kočovská et al. (2012)
18	Increased VLCFA PE lipids	Dayan Goodenowe	2009	Pastural et al. (2009)
19	Decreased biopterin	Richard Frye	2010	Frye et al. (2010)
20	Decreased plasma biotin	James Adams	2011	Adams et al. (2011)
21	Decreased plasma ATP, increased adenosine	James Adams	2011	Adams et al. (2011)
22	Increased plasma glutamate	James Adams	2011	Adams et al. (2011)
23	Decreased branched chain amino acids	James Adams Rabindra Tirouvanziam Joe Gleeson	2011, 2012	Adams et al. (2011)Novarino et al. (2012)Tirouvanziam et al. (2012)
24	Increased plasma and urine oxalate	Jerzy Konstantynowicz	2012	Konstantynowicz et al. (2012)
25	Propiogenic amino acid metabolism	Derrick MacFabe, M. Al-Owain	2007, 2012	Al-Owain et al. (2013); MacFabe et al. (2007)
26	Decreased carnitine synthesis	Art Beaudet	2012	Celestino-Soper et al. (2012)
27	Eicosanoids	Afaf El-Ansary	2012	Beaulieu (2013); El-Ansary and Al-Ayadhi (2012); Gorrindo et al. (2013)
28	Oxidative shielding and metabolic memory	Robert Naviaux	2012	Naviaux (2012b)
29	Decreased fatty acid oxidation	Richard Frye	2013	Frye et al. (2013b)
30	Decreased plasma choline and betaine	Jill James	2013	Hamlin et al. (2013)
31	Increased rT3/TSH	Richard Frye	2017	Frye et al. (2017)
32	Cell danger response metabolism	Robert Naviaux	2012 2013 2014 2015 2017	Naviaux et al. (2014); Naviaux et al. (2015); Naviaux (2012a, 2014); Naviaux et al. (2017); Naviaux et al. (2013)

persistence of these traits can be driven by durable epigenetic changes that trace to mitochondrial function (Minocherhomji et al., 2012; Naviaux, 2008; Wallace and Fan, 2010), but produce changes in gene expression that can persist beyond their utility because of time lags between frequent activation in a harsh environment, and recovery. Under these changing environmental conditions, mixtures of ecoalleles and epigenetic changes that were once advantageous may become a disadvantage. When this happens in ASD, cofactor and metabolic therapies directed at ecoallele-driven phenotypes in ASD can help strengthen resilience in the homeostatic safety net and improve behavioral symptoms (Frye et al., 2013a, 2016b).

10. Metabolism and ASD behavior

The idea that behaviors in autism are caused by a change in metabolism is not new. The first organic abnormalities reported in ASD were metabolic (Rimland, 1964; Sutton and Read, 1958) (Table 1). Several genetic disorders of purine and pyrimidine (Micheli et al., 2011; Nyhan et al., 1969; Page and Coleman, 2000) and energy metabolism (Stockler-Ipsiroglu and van Karnebeek, 2014) are associated with autistic behaviors. Bernie Rimland, the founder of the Autism Research Institute (ARI), pioneered the metabolic approach to treatment in the first clinical trial of pyridoxine in children with ASD (Rimland et al., 1978). When cells detect genetic or environmental threats, mitochondrial function changes in a predictable way. These changes produce the CDR and act as a two-edged sword. On the one hand, the change in cell metabolism allows threatened cells surrounding the injury to better survive dangerous conditions. On the other hand, when these changes become widespread and occur during pregnancy or the first 3 years of life, and are severe or sustained, they can alter the trajectory of normal

child development. The CDR produces effects on purinergic signaling that change how neural circuits are selected and how synapses are formed and pruned in the brain (Sipe et al., 2016). Many other organ systems are also affected by the CDR. These include the immune system, the gut microbiome, and the autonomic nervous system. Each of these is documented to be dysfunctional in autism.

Over 30 metabolic abnormalities have been described in ASD over the past 60 years (Table 1). All are known markers of the cell danger response (Naviaux, 2014; Naviaux et al., 2016). Interestingly, this set of about 30 different metabolic pathways is shared with the conserved cellular response to danger or threat regardless of whether the trigger was a virus (Wikoff et al., 2009), a bacterium (Degtyar et al., 2009), genetic forms of mitochondrial disease (Nikkanen et al., 2016), or neurodevelopmental disorders with complex gene-environment pathogenic mechanisms like autism (James et al., 2004). The hopeful message behind the CDR hypothesis is that the root cause of the communication difficulties, social anxiety, sensory abnormalities, GI problems, seizures, allergies, and many other comorbidities in autism, is a treatable metabolic syndrome. This means that contrary to classical teaching in medical schools around the world for the past 70 years, autism may not be permanent in some children. By treating the root cause, the CDR hypothesis gives hope that longstanding roadblocks to development can be removed and the children can make remarkable progress, despite a great heterogeneity in the causes of ASD. Future clinical trials will be needed to test this hypothesis rigorously.

11. Purinergic signaling and ASD

11.1. Purinergic signaling maintains the CDR

If the CDR is the problem, what is the cellular signal that keeps it turned on after it is no longer needed for healing? To answer this question, researchers had to weave together several apparently unrelated threads. These threads included research on mitochondria and healing (Naviaux et al., 2009), genetic forms of mitochondrial dysfunction in autism (Graf et al., 2000), genetic forms of autism associated with increased purine metabolism (Nyhan et al., 1969), and the paradoxical improvement with fever that proved that the core behaviors of autism could be dynamically regulated by metabolism (Curran et al., 2007). The author hypothesized that the root cause of pathological persistence of the CDR was continued excessive or unbalanced purinergic signaling, called hyperpurinergia (Naviaux et al., 2013), and dyspurinergia, respectively.

Hyperpurinergia is a universal and normal feature of the immediate and subacute cellular response to injury. Stressed cells release ATP and other small molecules less than about 800 Da in size through specialized membrane channels. The pannexin/P2X7 porin is an example of one of these stress-gated channels (Burnstock and Knight, 2017; Naviaux, 2012b). This phenomenon is illustrated in the whiteboard animation available at: https://www.youtube.com/watch?v=zIdUufy8Lks. When ATP, UTP, and other mitokines (signaling molecules traceable to mitochondria) are released through the stress-gated channels in the cell membrane, they bind to receptors on the cell surface to signal danger. Nineteen (19) purinergic receptors have been cloned. There are 8 P2Y receptors, 7 P2X receptors, and 4 P1 (adenosine) receptors. Extracellular ATP, ADP, adenosine, and UDP-glucose are important regulators of mast cell degranulation (Lazarowski and Harden, 2015; Osipchuk and Cahalan, 1992), neutrophils and T-cell function (Ledderose et al., 2015). Many different disease processes are regulated by purinergic signaling (Burnstock, 2014). Once the danger has passed, the release of ATP decreases, the CDR turns off, cells can complete the healing cycle, and return to normal “peacetime” function. Mixtures of CDR triggers can be synergistic. This contributes to the concept of total toxic load. Sequential exposures during critical developmental windows can stack to create a “perfect storm” of events that can derail the healing process and lead to pathological persistence of the CDR.

11.2. Nucleotide metabolism regulates purinergic signaling

In principle, hyperpurinergia can be produced by increased release of ATP and related receptor ligands, increased nucleotide dwell time caused by decreased metabolism or inactivation of purinergic effectors, a failure of receptors to desensitize once their job is done, or a combination of each. The dwell time of extracellular nucleotides in the pericellular halo is tightly regulated by cell-specific expression of CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5′-nucleotidase) on the cell membrane. These proteins convert extracellular ATP and ADP to AMP (CD39), and AMP to adenosine (CD73) in calcium- and magnesium-dependent reactions (Antonioli et al., 2013) (Fig. 1). Adenosine is then either taken up by the cell through the equilibrative nucleoside transporter 1 (ENT1, SLC29A1), or metabolized to inosine by adenosine deaminase (ADA). Inosine is hydrolyzed to yield hypoxanthine and ribose-1-phospate by purine nucleoside phosphorylase (PNP). PNP does not accept adenosine as a substrate and is not a source of adenine. Adenine base can be produced by methylthioadenosine phosphorylase (MTAP) during polyamine synthesis and methionine salvage (Mavrakis et al., 2016). Free adenine can be released or taken up by cells via the equilibrative nucleobase transporter (ENBT1, SLC43A3). When taken up, adenine is used for salvage synthesis of AMP by adenine phosphoribosyl transferase (APRT). Hypoxanthine can be taken up by cells for salvage synthesis of purines via membrane transporters like the concentrative sodium-dependent nucleobase transporter 1 (SNBT1, SLC23A4), then condensed with phosphoribosylpyrophosphate (PRPP) by the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) to make IMP. Hypoxanthine can also be oxidized in the extracellular space to xanthine and uric acid, with the production of superoxide (O_2^{-}) by the molybdenum-dependent flavoprotein xanthine oxidase (XO). Extracellular superoxide is converted to hydrogen peroxide (H_2O_2) by the copper and zinc-dependent, extracellular superoxide dismutase (SOD3). Extracellular and intracellular NAD^+ and $NADP^+$ are converted by CD38 to cADPR and NAADP, respectively. Both cADPR and NAADP activate calcium influx via the TRPM2 membrane channels. Inside the cell, cADPR releases calcium from the ER through the ryanodine receptor (RyR), and NAADP releases acidic calcium stores from lysosomes through the two pore channel (TPC) proteins. In anti-inflammatory, M2 polarized mitochondria the flux of NAD^+ and NADPH through the nicotinamide nucleotide transhydrogenase (NNT) favors NADH and $NADP^+$ production. In M1 (pro-inflammatory) mitochondria, the flux through NNT favors NADPH and NAD^+. NADPH and oxygen are then used by mitochondrial outer membrane-associated NADPH oxidase 4 (NOX4) to produce H_2O_2. Uric acid is transported into the cell by the cytokine-regulated uric acid transporter SLC2A9 (So and Thorens, 2010) and can stimulate inflammation directly by triggering the assembly of the NLRP3 inflammasome (Ghaemi-Oskouie and Shi, 2011) (Fig. 1).

UTP, UDP, and UDP-glucose are also released from cells under stress and act as signaling molecules that bind to purinergic receptors. CD39 and CD73 can also dephosphorylate UTP and UDP to produce extracellular uridine. Uridine can be metabolized by uridine phosphorylase (UPase) to produce the free nucleobase uracil and ribose-1-phosphate. Uracil can be imported as a free nucleobase into the cell by SNBT1. Uridine is transported into the cell through ENT1. Inside the cell, uridine is salvaged by phosphorylation by uridine-cytidine kinase 1 and 2 (UCK1/2). By regulating the relative expression of CD39, CD73, ADA, ENT1, SNBT1, ENBT1, PNP, XO, SOD3, CD38, TRPM2, APRT, HGPRT, SLC2A9, and UCK1/2, the nuanced informational content of the unstirred water layer (UWL) produced by the release of extracellular ATP and UTP can be precisely calibrated in accordance with the functional states of each responding cell type (Fig. 1).

11.3. Purinergic signaling, the CDR, and the symptoms of ASD

When healing is incomplete, cells can be left in a state of hyper- or hypo-responsiveness to new threats. Chronic changes in purinergic signaling alter pain perception (Magni et al., 2017), and the processing of other sensory stimuli (Breza and Travers, 2016; Dietz et al., 2012). This is not unlike a cellular form of post-traumatic stress disorder (PTSD) resulting in durable changes in behavior after exposure to a transient, but serious stress. Cells cannot heal if a significant fraction of ATP is exported for purposes of signaling danger instead of being kept in the cell for normal energy metabolism. The use of antipurinergic drugs like suramin to treat a misfiring CDR has been called "molecular armistice therapy" because it sends a signal that "the war is over". This decreases losses of ATP through stress-gated membrane channels, and decreases purinergic autocrine and paracrine signaling of danger (https://www.youtube.com/watch?v=zIdUufy8Lks) so cells and mitochondria can return to peacetime metabolism needed for healing and development. The concept that purinergic signaling abnormalities are involved in ASD and can alter behavior is not just theoretical. Evidence of purinergic signaling abnormalities was found in children with ASD in a recent gene expression study (Ginsberg et al., 2012). Purinergic signaling has been shown to regulate a number of the cellular comorbidities and functional abnormalities associated with ASD (Table 2).

12. Preclinical studies of antipurinergic therapy

The idea that purinergic signaling might be involved in autism was born in 2008 (Naviaux research supported by Mr. Dan Wright). In 2010, the Naviaux Lab received a "Trailblazer" award from Autism Speaks (https://autismspeaksblog.wordpress.com/tag/mitochondria/) to test this idea. Suramin has many actions (Liu and Zhuang, 2011; Voogd et al., 1993). One of its most studied actions is as a non-selective purinergic antagonist (Burnstock, 2006a). The 2013 paper (Naviaux et al., 2013) describing the results of the first suramin treatment studies in ASD mouse models showed that abnormal persistence of extracellular ATP signaling could cause ASD-like behaviors. It also produced excitoxicity that led to the death of Purkinje cells in the cerebellum. Rebalancing the CDR with suramin restored normal behavior and prevented the loss of these cells (Naviaux et al., 2013). Two additional studies confirmed that antipurinergic therapy with the non-selective purinergic inhibitor suramin improved both the core behaviors and the metabolic syndrome underlying autism-like symptoms in both the Fragile X genetic model and the environmental maternal immune activation (MIA) models (Naviaux et al., 2014; Naviaux et al., 2015). The mouse models also showed that high doses were not necessary. Low-dose suramin that produced blood levels of just 5–15 μM was both safe and effective in treating the symptoms of autism in these models.

13. Results of the SAT1 clinical trial

13.1. Metabolic abnormalities in ASD were improved by low-dose suramin

Using mass spectrometry, the metabolic pathways that were disturbed at baseline and changed by suramin were characterized in two mouse models of ASD-like behavior. Suramin treatment improved 17 of 18 (94%) biochemical pathways that were abnormal in the MIA mouse model (Naviaux et al., 2014) and 20 of 20 (100%) pathways disturbed in the Fragile X mouse model (Naviaux et al., 2015). Metabolomic analysis was also performed in the 10 children with ASD in the SAT1 study (Naviaux et al., 2017). This study showed that 21 of 28 (75%) of the pathways disturbed in children with autism were also abnormal in the mouse models. These included improvements in purines, 1-carbon/folate, S-adenosylmethionine (SAMe), glutathione, microbiome, branched chain amino acids, fatty acid metabolism, and others (Naviaux et al., 2017) (Fig. 2). These improvements in metabolism were associated with similar improvements in each of the core symptoms of autism. There was a flowering of interest in social communication, new language, new social activities on the playground like playing tag, and at home like playing catch and other games with neurotypical siblings. The half-life of suramin after a single-dose was 14.7 ± 0.7 days. Studies in African sleeping sickness have shown that the plasma half-life of suramin can increase to 1 or 2 months after multiple doses (Hawking, 1940). As the single dose of suramin in the SAT1 study gradually wore off over 5–8 weeks, metabolism drifted back toward baseline, and most of the behavioral gains were lost. It is not yet known if regular suramin given every month or so could support continued developmental gains. These studies strongly underscore the hopeful message that the symptoms of autism might be caused by a treatable metabolic syndrome and that antipurinergic therapy with low-dose suramin is a powerful tool in treating these fundamental metabolic abnormalities.

Table 2
Cellular comorbidities and functional abnormalities in autism spectrum disorder regulated by purinergic signaling.

No.	Feature	References
1	Innate immunity, allergies, and inflammation	West et al. (2011)
2	Autoimmunity	Savio and Coutinho-Silva (2016)
3	Microglial and astroglial activation	Butt (2011)
4	T-cell proliferation	Yu et al. (2010)
5	Th17 cells	Sullivan et al. (2014)
6	Treg cells	Cortes-Garcia et al. (2016)
7	NK cells	Raskovalova et al. (2005)
8	Mast cell activation	Feng et al. (2004)
9	Eosinophil activation	Ferrari et al. (2006)
10	Neuronal migration	Liu et al. (2008)
11	Brain injury and repair	Burnstock (2016)
12	Glutathione, ROS, and redox	Zhang et al. (2005)
13	Nitric oxide synthesis	Silva et al. (2006)
14	Apoptosis	Dawicki et al. (1997)
15	Gliovascular coupling	Pelligrino et al. (2011)
16	Synaptogenesis and neuronal plasticity	Pankratov et al. (2009)
17	cMet/mTor signaling	Gerasimovskaya et al. (2005)
18	PI3/AKT signaling	Katz et al. (2011)
19	Sensory perception and sensory integration	Pain (Burnstock, 2006b) Sight (Housley et al., 2009) Sound (Housley et al., 2002) Touch (Wang et al., 2010) Taste (Huang et al., 2009) Smell (Housley et al., 2009) Vestibular (Lee et al., 2001)
20	Epilepsy and the seizure threshold	Dona et al. (2009)
21	Oxytocin and vasopressin secretion	Song et al. (2009)
22	Obsessive compulsive behaviors	Mastrangelo et al. (2012)
23	Depression and affect	Sperlagh et al. (2012)
24	Cortisol secretion and the HPA axis	Bjelobaba et al. (2015)
25	Appetite and feeding behaviors	Stojilkovic (2009)
26	Anxiety and retention of aversive memories	Campos et al. (2014)
27	Self-injurious behavior	Mastrangelo et al. (2012)
28	Gut Inflammation and the microbiome	Estrela and Abraham (2011)
29	Gut permeability	Matos et al. (2007)
30	Food allergen reactivity	Leng et al. (2008)
31	GI motility, constipation, diarrhea, irritable bowel	Jimenez et al. (2014)
32	Parasympathetic autonomic nervous system	Passamani et al. (2011)
33	Blood pressure control	Pijacka et al. (2016)
34	Sleep	Halassa (2011)
35	Stem cell development	Burnstock and Ulrich (2011)

13.2. Core symptoms of ASD were improved by low-dose suramin

Low-dose of suramin used in the SAT1 study (Naviaux et al., 2017) improved the core symptoms of ASD measured by ADOS2 (autism diagnostic observation schedule, 2nd edition) score by 1.6 ± 0.55 points in 6 weeks ($p < 0.0028$; Fig. 3). Language, social interaction,

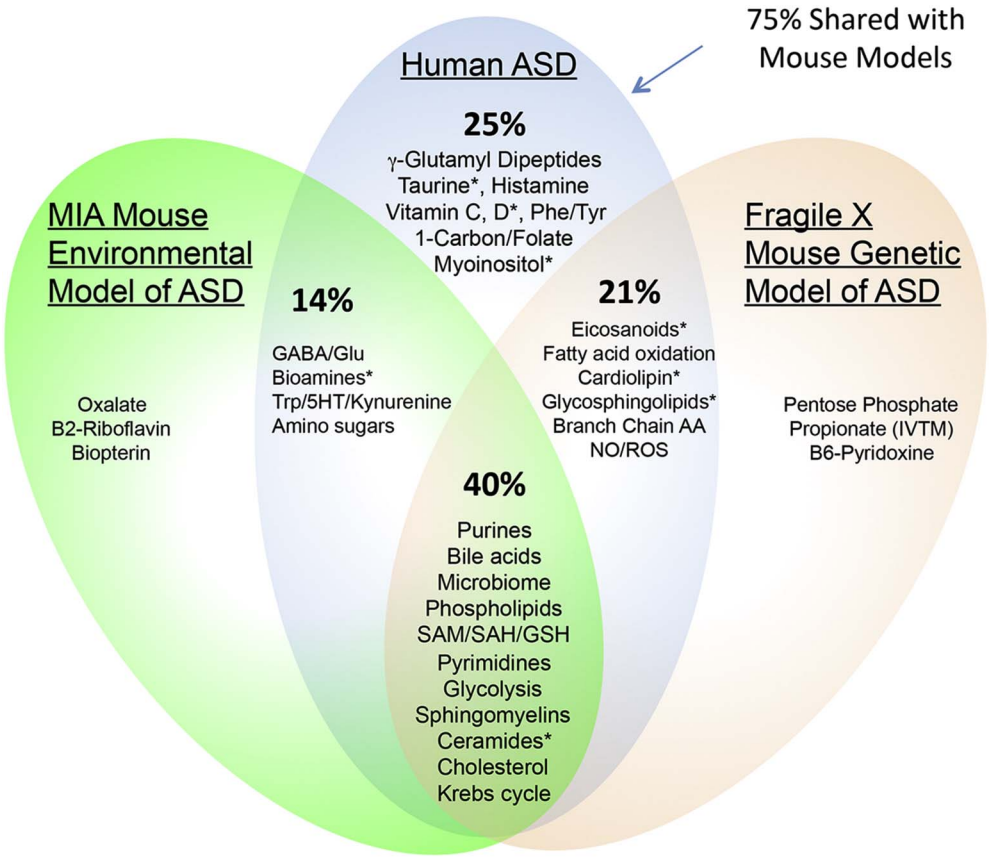

Fig. 2. Biochemical abnormalities of autism were improved by low-dose suramin. The figure lists the pathways that were improved by anti-purinergic therapy with suramin in children with ASD in the SAT1 study. Twenty-one of 28 (75%) of the metabolic pathway abnormalities were also improved by suramin in the maternal immune activation (MIA) and Fragile X mouse models of autism. Each of these pathways is known to play a role in the cell danger response (CDR). The observation that the core symptoms of ASD were improved with the metabolic pathways known to be associated with the CDR, supports the hypothesis that the behavioral symptoms of autism may be caused by a treatable metabolic syndrome.
Adapted from (Naviaux et al., 2017).

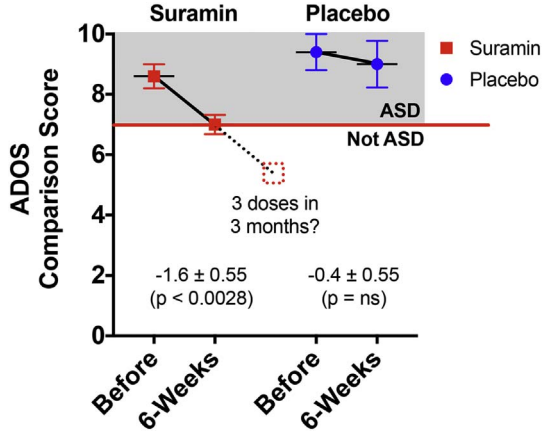

Fig. 3. A single dose of suramin Improved ADOS scores by 1.6 points in 6 weeks. ADOS2 comparison scores of 7–10 (gray box) are used as a gold standard for the diagnosis of autism spectrum disorder (ASD) and classical autism. A single dose of suramin resulted in a 1.6 ± 0.55 point improvement in ADOS scores, from a mean of 8.6 ± 0.9, to 7.0 ± 0.7 ($p < 0.0028$) when measured after 6 weeks. If a few doses of suramin given over 3 months produce improvements at the same rate of 1 point/month, then some children would be able to come off the autism spectrum (dotted red box), and those with more severe forms of autism might improve significantly. [Data from the SAT1 study (Naviaux et al., 2017); N = 5 per group.]

restricted interests, and repetitive movements all improved. Two children who were previously non-verbal spoke their first sentences. Suramin treatment was synergistic with regular school, educational enrichment programs, applied behavioral analysis (ABA), speech, and occupational therapy. None of these improvements were observed in the placebo group. The authors reported that the maximum benefit from a single dose of suramin occurred after 3 weeks then decreased slowly. Even after 6 weeks, three children had improved by 2 points and two children improved by 1 point, compared to baseline. No children were unimproved in the treatment group. In comparison, three children who received placebo were unchanged, and two improved by 1 point each. This gave rise to an estimate of the placebo effect of 0.4 ± 0.55 points (Fig. 3; p = 0.18; ns). ADOS comparison scores of 7–9 are used as a gold standard for the diagnosis of autism spectrum disorder (ASD). An ADOS score of 10 meets criteria for classical autism. If a few doses of suramin given over 3 months produce improvements at the same rate of 1 point per month, then children with symptoms that were initially severe enough to be on the spectrum (ASD = ADOS comparison scores of 7–9), might be able to come off the autism spectrum (dotted red box), and those with more severe forms of autism might improve significantly (Fig. 3). Does this mean suramin might be the first effective drug treatment for autism in nearly 75 years of research efforts? It is too early to say. Some biomedical treatments of ASD show benefits for a few weeks or months, then lose effectiveness over time. There is no evidence this could happen with suramin, but more clinical trials are needed. We need to know if a few doses given over a few months are safe and are able to maintain the same rates of ADOS score improvement of about 1 point per month of treatment. If this is true, then we are one step closer to the goal.

13.3. Low-dose suramin safety

Low-dose suramin that produced blood levels of just 5–15 μM, was safe and produced significant improvement in ASD symptoms at 6 weeks in the SAT1 trial (Naviaux et al., 2017) (Fig. 4). This low dose of suramin has never been studied before. The side effect profile of high-dose suramin (150–270 μM) is known from cancer chemotherapy studies (Stein, 1993). One author has expressed concern about the safety of high-dose suramin (Theoharides, 2013) citing a review of cancer studies (Kaur et al., 2002) that used prolonged exposure to high-doses of suramin that produced 25-times higher blood levels than those needed

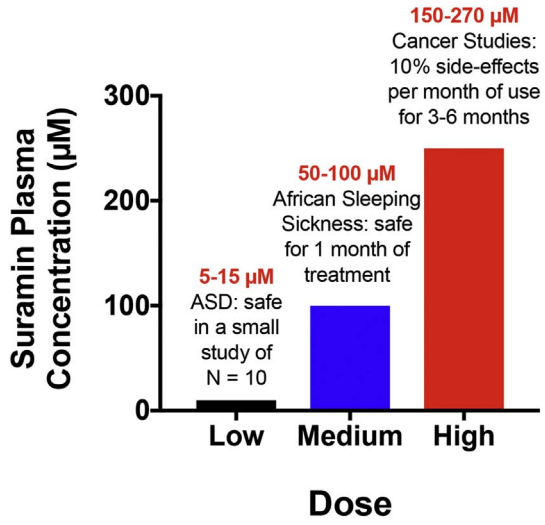

Fig. 4. Low-dose suramin was safe in children with autism spectrum disorder (ASD). The safety and toxicity of suramin are dose-dependent. Definitions: Low-dose suramin (5–15 μM) as antipurinergic therapy of ASD, Medium-dose (50–100 μM) as antimicrobial therapy for trypanosomiasis, high-dose (150–270 μM) as anticancer adjunct therapy.

to treat autism (Naviaux et al., 2017). Cancer studies typically used a dose and schedule of suramin designed produced blood levels of 250 μM compared to 10 μM used in the SAT1 study. High doses of most drugs have toxicities that are not seen at lower doses. The safety and side effect profile from medium-dose suramin (50–100 μM) is well known from nearly 100 years of study in African sleeping sickness (Hawking, 1940, 1978). This work showed that when a cumulative dose of 2.5–3 g/m^2 was divided into five, weekly intravenous infusions over a month, the elimination half-life increased from 2 weeks to 1.5–2 months, and suramin concentrations ≥ 4 μM (≥ 5 mg/L; MW = 1297 g/mol) were safely maintained for at least 6 months. About 10% of patients treated were rapid excretors and did not maintain these concentrations for as long (Hawking, 1978). These studies were done in Africa before the era of pharmacogenomics, so future studies in more ethnically diverse patient populations may reveal genetic differences in the handling and response to suramin that cannot yet be predicted. The side effect profile of low-dose suramin, given for several months is unknown. Future studies are needed to answer four big questions: 1) Do all children with ASD benefit, or just a fraction? 2) Does suramin lose effectiveness after a few months, or do children continue to benefit for as long as the drug levels in the blood are above 5 μM? 3) Does suramin need to be given for life, or are 4–8 doses over 6–12 months sufficient for normal child development to become self-sustaining? and 4) How long can low-dose suramn be used safely?

14. Sparking a Renaissance in drug development

14.1. Suramin as the first antipurinergic drug

Like the first antibiotic, or first beta-blocker for high blood pressure, suramin is the first antipurinergic drug (APD). APDs represent a class of medicines that is completely new to the world's pharmacopeia. Soon other drugs that work like suramin will be developed (Jacobson and Müller, 2016). Eventually, the goal of this new Renaissance would be to create a shelf-full of APDs, each with slightly different pharmacologic properties that would allow doctors to pick and choose the best match for each patient. The recent discovery that suramin prevents Zika, Ebola, Chikungunya, Coxsackievirus A16, and Enterovirus A71 (Albulescu et al., 2017; Henss et al., 2016; Ren et al., 2017) from infecting cells may prompt additional clinical trials and further interest in APD development. Currently however, suramin is the only non-selective APD available for human use. Research into the role of purinergic signaling in autism is so new that we do not yet know which of the 19 purinergic receptors are most relevant. Suramin is a broad-spectrum inhibitor of most purinergic signaling systems.

Several P2Y12-selective antagonists like Plavix (clopidogrel) are used as antiplatelet agents to prevent blood clots, strokes, and heart attacks. However, their safety and activity in autism is unknown. Brilliant Blue G (BBG) is a P2X7 inhibitor and protein binding dye that has been used successfully in animal models to prevent excessive inflammation after spinal cord injury (Peng et al., 2009), Parkinson disease (Ferrazoli et al., 2017), and acetaminophen-associated liver injury (Abdelaziz et al., 2017). BBG is also widely used by ophthalmologists during retinal surgery (Azuma et al., 2016). Several experimental APDs are in clinical trials for rheumatoid arthritis and pain that target the P2X7 receptor, but none are yet available to prescribing physicians. It is likely that novel antipurinergic activities will be found in herbs, fungi, and other natural products distributed throughout the biosphere (Faria et al., 2012; Soares-Bezerra et al., 2013). Some may already be in use but their essential pharmacologic action as purinergic inhibitors is not yet known. Alternatively, knowledge of the synthetic chemical additives in our food chain that activate or inhibit purine and pyrimidine signaling (Ferreira et al., 2016), may shed new light on why some food colorings and additives are a problem in some children (Weiss, 2012).

14.2. Current clinical trials of antipurinergic drugs

In 2017, a search of the keyword "purinergic" among interventional trials in clinicaltrials.gov returned 418 studies in the US. Currently, over 90% of these studies are focused on platelets and heart disease. However, the broad involvement of purinergic signaling in nearly every chronic disease in which it has been studied (Burnstock, 2017) suggests great potential for the development of this new class of medications.

15. Conclusions

Over $1 billion has been spent on genetic research in autism over the past 10 years by the NIH, Autism Speaks, and the Simons Foundation. This work has shown that hundreds of genes play a role in different children, and that no single gene accounts for more than 1–2% of autism (Talkowski et al., 2014). While most genetic studies conclude that each genetic cause of ASD must be treated differently, the CDR hypothesis suggests that one mechanism—a unified cellular response—might be at the root of all the different causes of autism. In addition, the CDR hypothesis comes with a detailed molecular mechanism and a treatment. This new theory has already been rigorously tested in the lab since 2011 and found successful in classical animal models of autism. It also showed promise in 10 children with ASD as a unifying theory of pathogenesis in the SAT1 clinical trial (Naviaux et al., 2017).

15.1. The economic impact of a treatment for ASD

The average family caring for a child with ASD in the US spends over $17,000 annually in extra costs not covered by the health care and educational systems (Lavelle et al., 2014). About 3.5 million Americans today live with ASD (Buescher et al., 2014). Using the current estimates of 1 in 68 (Developmental Disabilities Monitoring Network Surveillance Year Principal et al., 2014) to 1 in 45 (Zablotsky et al., 2015) children in the US with ASD and a birthrate of 4 million children per year, about 75,000 ± 15,000 children will be diagnosed with ASD this year in the United States. The national economic cost of autism in the US is estimated to be $268 billion annually, or close to $75,000 per year per patient with ASD. This could rise to $461 billion by 2025 if the rising prevalence of ASD is not stopped (Leigh and Du, 2015). If a new treatment could help just 10% of patients come off the autism spectrum, it would bring back over $26 billion into the US economy each year ($268 billion × 10% = $26.8 billion). The financial return from this

discovery in a single year would be enough to support over 100 years of autism research funded by the National Institutes of Health (NIH). The 2016 budget for autism research projects at NIH was $232 million (https://report.nih.gov/categorical_spending.aspx;$26.8 billion saved ÷ $0.232 billion/year NIH budget = 115 years).

15.2. Human impact

If autism is proven to be a treatable metabolic syndrome in some children, it means that some children now living with disabling forms of ASD, whose parents fear might never be able to live independently, could have a chance for independence and live happy, self-reliant lives. In addition, ASD often affects children who have shown early gifts and might otherwise grow up to become some of the best and brightest of their generation. If new science can lift their disabling symptoms without touching the unique gifts that make them special, the children with ASD today could grow up to become the young men and women of tomorrow who can think creatively to crack the problems that no one else can—to solve our technological problems, to ease social unrest, protect the environment, and help create a healthier future for us all.

16. Special note from the author

Until recently, the public has not heard the name of a new drug until it has been approved by the FDA to treat a particular disorder and it is ready to be marketed by its manufacturer. This has the beneficial effect of protecting patients from asking for, or trying a drug that has not yet been proven safe and effective for their disease. A growing number of drugs are experiencing new interest as researchers discover new uses for old drugs. This is called “drug repurposing”. Drug repurposing is not as simple as using an old drug “off-label” to treat a new disorder. Many drugs are not safe or effective when used this way. Many times the dose and schedule, and even the fundamental pharmacology, like the half-life and volume of distribution, of a drug are different in different patient populations. Careful clinical trials are needed to establish safety and efficacy for each new indication.

Suramin is approved to treat African sleeping sickness (trypanosomiasis). It is not approved for the treatment of autism. There is currently no approved use of suramin in the United States. It is illegal to import suramin into the US for human use without FDA approval. Like many intravenous drugs, when administered improperly by untrained personnel, at the wrong dose and schedule, without careful measurement of drug levels and monitoring for toxicity, suramin can cause harm. Careful clinical trials will be needed over several years at several sites to learn how to use low-dose suramin safely in autism, and to identify drug-drug interactions and rare side effects that cannot currently be predicted. The author strongly cautions against the unauthorized use of suramin. Ultimately, clinical trials may show that suramin is not the final answer for autism. Its effects may be limited to a small number of children, or they may not last, or side effects may emerge. However, the discovery that the cell danger response and purinergic signaling are fundamental features of ASD is now stimulating new research around the world. New antipurinergic drugs, and the rediscovery of old ones, will not be far behind.

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Conflicts of interest

RKN is a scientific advisory board member for the Autism Research Institute (ARI) and the Open Medicine Foundation (OMF), a consultant for Stealth Biotherapeutics, and has submitted a technology disclosure to UCSD describing antipurinergic therapy for autism and related spectrum disorders.

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