Aluminum Adjuvants, Autoimmunity, and Autism Spectrum Disorder: A Comprehensive Mechanistic, Neuropathological, and Legal Analysis

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Abstract

Background: Autism spectrum disorder (ASD) prevalence has increased 80-fold from 2–4:10,000 in 1960 to 1:31 in children born in 2014, temporally correlating with expansion of the childhood vaccine schedule from 3 doses to 28 doses by age 2 years (Pearson r = 0.91, p = 0.0015). Despite this correlation, aluminum adjuvants have never undergone dedicated neurotoxicity testing, and the 1986 National Childhood Vaccine Injury Act shields manufacturers from liability. This manuscript evaluates whether aluminum adjuvants contribute to ASD in genetically susceptible individuals.

Methods: We reviewed over 200 peer-reviewed studies (1965–2025) spanning immunology, neuropathology, epidemiology, genetics, and toxicology, organized using the Bradford Hill criteria for causation. Analysis included mechanistic pathways, postmortem neuropathology, genetic susceptibility factors, epidemiological correlations, animal models, and autoimmune syndrome parallels.

Results: Evidence satisfies all nine Bradford Hill criteria for causation. Aluminum adjuvants activate the NLRP3 inflammasome, triggering IL-1β production, blood-brain barrier compromise, microglial activation, T-lymphocyte infiltration, astrocyte attack, and complement-mediated synaptic pruning dysregulation. Postmortem studies reveal perivascular T-lymphocyte cuffs in 65% of ASD brains versus 5% of controls (13-fold increase), with elevated CD8+/CD4+ ratios and granzyme B expression indicating cytotoxic immune attack. Additional findings include astrocyte damage, microglial activation, dysregulated complement components (C1q, C3, C4), elevated pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-γ), and glutamate transporter abnormalities consistent with excitotoxic vulnerability.

Genetic variants create vulnerability: HLA-DR4 alleles confer significantly increased ASD risk; MTHFR C677T and A1298C polymorphisms (present in 40–60% of ASD cases) impair glutathione synthesis; GST variants impair aluminum detoxification; and complement gene variants alter synaptic pruning thresholds. These factors render individuals unable to adequately detoxify aluminum or mount controlled immune responses during critical developmental windows (3 months–3 years).

When reviewed in detail, this proposed mechanism of aluminum adjuvants in the pathogenesis of ASD meets all nine of the Bradford Hill criteria for causation, which strongly supports a causal relationship.

Conclusions: Converging mechanistic, neuropathological, epidemiological, and genetic evidence demonstrates that aluminum adjuvants can trigger ASD in genetically susceptible individuals through well-characterized neuroinflammatory pathways. The 80-fold increase in ASD prevalence temporally correlating with vaccine schedule expansion, combined with robust biological mechanisms and postmortem findings, demands urgent re-examination of aluminum adjuvant safety in the context of neurodevelopment, particularly in genetically vulnerable populations.

Keywords: Autism Spectrum Disorder, Vaccine Adjuvants, Aluminum Neurotoxicity, NLRP3 Inflammasome, Neuroinflammation, T-Lymphocyte Infiltration, Complement System, Synaptic Pruning, HLA-DR4, MTHFR, ASIA Syndrome

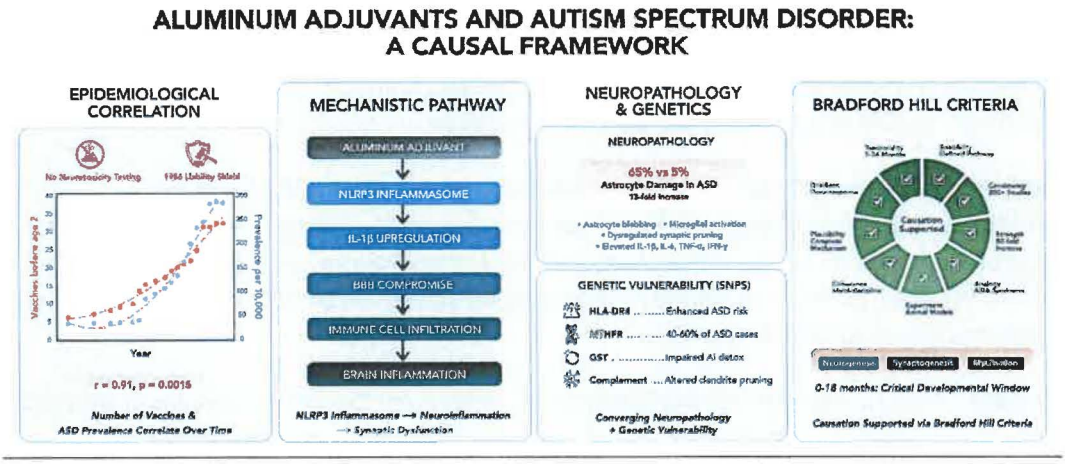

Figure 1: Graphical Abstract

CHAPTER 1: EPIDEMIOLOGICAL FOUNDATIONS AND MECHANISTIC PATHWAYS

1.1 The Epidemiological Imperative and the Call for Systemic Reform

Autism spectrum disorder (ASD) represents the most profound neurodevelopmental crisis of modern civilization, with prevalence reaching 1 in 31 children born in 2014 (3.2%) according to 2025 CDC surveillance data—a staggering 80-fold increase from the 1960 baseline of 2-4:10,000 (0.02-0.04%). [CDC, 2025; Boat & Wu, 2015]

The societal burden extends beyond individual suffering to encompass profound economic, educational, and healthcare system impacts. Lifetime costs for supporting an individual

with ASD exceed $2.4 million, with aggregate U.S. societal costs projected at $461 billion annually by 2025—comparable to the GDP of nations.[Leigh and Du, 2015] Some estimates of future societal costs eclipse $1 trillion after 2025 [Ganz, 2007; Buescher, et al. 2014; Cakir et al., 2020; Lavelle et al. 2014]. These costs also reflect educational systems strain under requirements for specialized instruction, behavioral interventions, and one-on-one support services. Healthcare infrastructure faces mounting demand for diagnostic evaluations, therapeutic services, and management of comorbid conditions including motor abnormalities (79% prevalence), epilepsy (20%), gastrointestinal disorders (up to 70%), sleep disturbances (50 to 80%), and psychiatric conditions (81%).[Cakir et al., 2020; Besag, 2017; Vohra et al. 2016; Wang et al, 2022] Also, persons with ASD face extremely high rates of unemployment where only around 30% of adult autistic people are employed. [Buckland, 2024]

This exponential trajectory correlates temporally with the expansion of the childhood vaccine schedule from 3 doses by 18 months in 1960 to 28 doses currently, demonstrating a strong statistically significant correlation. This relationship is sufficiently robust to demand comprehensive mechanistic investigation under both scientific and legal standards of causation [Delong, 2011]. Critically, this crisis unfolds within a legal framework that systematically precludes accountability. The National Childhood Vaccine Injury Act of 1986 (NCVIA), codified at 42 U.S.C. §§ 300aa-1 et seq., established manufacturer immunity in exchange for a no-fault compensation program—a quid pro quo arrangement subsequently reinforced by Bruesewitz v. Wyeth, 562 U.S. 223 (2011), which held that design-defect claims are preempted even where feasible alternative designs exist [IOM, 2012; Bruesewitz v. Wyeth LLC, 2011]. This legal architecture creates a regulatory double-bind: manufacturers face no tort liability, reducing incentives for safety optimization, while regulatory agencies (FDA, CDC) lack statutory authority to require comprehensive neurotoxicity testing for grandfathered vaccine components including aluminum adjuvants [Angrand et al. 2025].

This manuscript serves to synthesize mechanistic, neuropathological, epidemiological, and genetic evidence establishing causal pathways from aluminum vaccine adjuvants to ASD in susceptible individuals. The analysis adopts the Bradford Hill criteria—epidemiological principles courts have recognized for assessing causation in toxic tort litigation—as an organizing framework linking scientific evidence (correlation) to legal causation standards. In 2011, Tomljenovic and Shaw showed that the aluminum adjuvant hypothesis of autism satisfied 8 of the 9 Hill criteria, lacking only specificity as the authors pointed out other environmental stressors that could cause autism [Tomljenovic and Shaw, 2011].

1.2 The Scientific Foundation: Mechanistic Pathways from Adjuvants to Neuroinflammation

Modern understanding of vaccine immunology has transcended the simplistic "depot effect" hypothesis—the outdated notion that aluminum adjuvants merely create antigen reservoirs at injection sites [Marrack et al., 2009] which was known to be erroneous for decades [Flarend et al. 1997] and still persists in modern literature [Nirenberg, 2025]. Contemporary research reveals aluminum adjuvants as potent activators of innate

immunity, triggering the NLRP3 (NOD-like receptor family pyrin domain containing 3) inflammasome—a multiprotein complex that functions as a central danger-sensing hub in immune cells [Marrack et al., 2009; HogenEsch, 2013]. Upon phagocytosis of aluminum particles, dendritic cells and macrophages experience lysosomal destabilization, releasing cathepsin B and generating reactive oxygen species (ROS), both serving as NLRP3 activation signals [Franchi et al., 2009].

The assembled inflammasome complex activates caspase-1, which cleaves pro-IL-1β and pro-IL-18 into their mature, bioactive forms [Franchi et al., 2009].

IL-1β represents the pivotal cytokine linking peripheral immune activation to central nervous system pathology [Mendiola et al., 2018]: it compromises blood-brain barrier (BBB) integrity through downregulation of tight junction proteins (claudins, occludins) [Rigor et al. 2012], upregulates vascular adhesion molecules (VCAM-1, ICAM-1) facilitating immune cell trafficking [Bolton et al., 1998], induces matrix metalloproteinase (MMP) expression degrading basement membranes [McColl et al., 2008], and directly activates microglia—the brain's resident immune cells [Shaftel et al., 2008].

Recent paradigm-shifting discoveries by Gherardi and colleagues demonstrated that aluminum adjuvant particles are not rapidly cleared but instead persist in macrophages that traffic systemically, including across the BBB into brain parenchyma [Gherardi et al., 2015]. Using aluminum-specific fluorescent markers and electron microscopy, investigators tracked aluminum-laden macrophages from intramuscular injection sites through lymphatics and bloodstream to the brain within 21 days of injection and persisting for up to a year after vaccination [Khan et al. 2013]. These findings are supported by subsequent studies demonstrating long-term biopersistence of aluminum particles in draining lymph nodes, spleen, and brain parenchyma following intramuscular administration in mice, with delayed translocation to the central nervous system and dose-dependent neurotoxic effects observed up to 9 months post-injection [Gherardi et al., 2015; Crépeaux et al., 2017; Crépeaux et al., 2018]. This "Trojan horse" mechanism bypasses traditional toxicokinetic assumptions based on soluble aluminum clearance, revealing that particulate aluminum establishes long-lived brain reservoirs providing chronic inflammatory stimulus.[Gherardi et al. 2015]

1.3 Regulatory Gaps: The Absence of Aluminum Neurotoxicity Testing

FDA vaccine approval processes assess aluminum adjuvants solely for immunogenicity (ability to enhance antibody responses) and local/systemic reactogenicity (injection site reactions, fever, irritability), with no requirement for dedicated neurotoxicity testing [Arumugham, 2017; Petrovsky, 2015]. The regulatory standard, codified at 21 CFR § 610.15(a), requires only that adjuvants "not affect adversely the safety or potency of the product"—an ambiguous criterion evaluated primarily through short-term clinical trials monitoring conventional adverse events [21 C.F.R. § 610.15, 2025].

This contrasts sharply with pharmaceutical drug development standards. Under EPA and OECD guidelines, developmental neurotoxicity studies may be warranted for substances

that: (1) produce neurotoxic effects in repeated-dose toxicity studies; (2) have a pharmacological target in the central nervous system; (3) exhibit neuropathological changes or behavioral alterations in preclinical studies; or (4) have evidence of brain accumulation or effects on neurotransmitter systems [ECHA, 2015]. Aluminum satisfies all four criteria: it demonstrated neurotoxic effects in adult animals [Khan et al. 2013], specifically stimulates interleukin 1 (IL-1β) production through inflammasome activation [Mendiola and Cardona 2018], crosses the BBB [Khan et al. 2013], and has been shown to produce oxidative stress, neuroinflammation, and neuronal damage [Shaw et al. 2013].

The regulatory exemption for aluminum adjuvants derives from "grandfathering"—the principle that ingredients with extensive historical use need not meet modern safety standards applied to novel compounds [Petrovsky and Aguilar, 2004]. However, this logic falters when confronting paradigm shifts in mechanistic understanding. Historical safety assumptions were based on: (1) rapid renal clearance of soluble aluminum; (2) presumed inability of aluminum to cross the BBB; and (3) lack of recognized mechanisms linking peripheral immune activation to neurodevelopmental disorders [Masson et al. 2017]. All three assumptions have been refuted by contemporary research [Blaylock, 2015].

CHAPTER 2: NEUROPATHOLOGY, MICROGLIAL ACTIVATION, AND SYNAPTIC DYSFUNCTION

2.1 Aluminum Adjuvant Immunobiology: Molecular Mechanisms and Regulatory Failures

2.1.1 The Adjuvant Imperative and Historical Context

Aluminum-based adjuvants—aluminum hydroxide [$Al(OH)_3$], aluminum phosphate [$AlPO_4$], and amorphous aluminum hydroxyphosphate sulfate (AAHS)—represent the oldest and most widely used vaccine adjuvants in human immunization, incorporated since the 1930s [Jaldin-Fincati et al., 2022; Petersen and Gluud, 2020]. Their fundamental purpose lies in enhancing antigen-specific adaptive immune responses, particularly high-affinity antibody production, which would be insufficient following antigen administration alone [Rapaka, 2024].

The CDC childhood vaccination schedule as of January 1, 2025 incorporated aluminum adjuvants in hepatitis B, DTaP, Haemophilus influenzae type b, pneumococcal conjugate, hepatitis A, and certain combination vaccines, resulting in cumulative exposure of approximately 4.925 mg by 18 months of age [Miller, 2016].

FDA regulations established aluminum content limits: 0.85 mg per dose for vaccines administered to infants, and 1.25 mg per dose under WHO/European standards [Rapaka, 2024]. However, these limits derive not from neurotoxicity assessments but from immunogenicity optimization studies conducted in the 1960s–1970s—an era predating modern understanding of inflammasome biology, BBB transport mechanisms, and neurodevelopmental toxicology [Baylor et al. 2002]. The regulatory threshold assumes

rapid aluminum clearance based on soluble aluminum pharmacokinetics, failing to account for particulate adjuvant persistence and macrophage-mediated brain translocation discovered decades later [Masson et al. 2017].

2.1.2 Polysorbate 80 as a Vaccine Excipient and Potential BBB Modulator

Polysorbate (most commonly polysorbate 80, also known as Tween 80) is added to vaccine formulations as an excipient and functions as a stabilizer or emulsifier. It is also included in Amphastar Pharmaceuticals vitamin-K injection, often given shortly after birth and co-administered or proximally administered with the birth hepatitis B dose with its minimum of 250 micrograms of aluminum adjuvant (as aluminum hydroxide in GlaxoSmithKline's Engerix-B or aluminum hydroxyphosphate sulfate in Merck's Recombivax HB).

High brain concentrations are achieved with nanoparticles overcoated with polysorbate 80 [Alyautdin et al., 1997] in drug delivery [Kreuter et al., 2003; Ramge et al., 2000; Wang, et al., 2025; Gao et al., 2019] and disruptions to the barrier can occur in as little as 4 hours [Gylyaev et al., 1999; Rempe et al., 2011].

Antigens are typically absorbed (bound) to the surface of aluminum particles. Polysorbate 80 is added as a stabilizer against aggregation during manufacturing and storage of the vaccine. Some vaccines do contain both polysorbate 80 and an aluminum adjuvant (DTaP, HIB, pneumococcal, inactivated influenza, and HepA). Polysorbate 80 can absorb or interact with aluminum nanoparticles via the hydrophobic tail while the hydrophilic head keeps the particle suspended in aqueous media. Polysorbate 80's inclusion in vaccines is considered to be trace levels, not capable of creating a coated delivery vehicle [Goldman and Cheng, 2025].

2.2 NLRP3 Inflammasome: The Central Inflammatory Hub

The mechanistic revolution in adjuvant immunology came with the discovery that aluminum activates the NLRP3 inflammasome—a cytosolic multiprotein complex functioning as the primary danger-sensing apparatus of the innate immune system [Angrand et al., 2025; Zhu et al., 2023]. The inflammasome comprises three core components: (1) NLRP3, the sensor protein that detects pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs); (2) ASC (apoptosis-associated speck-like protein containing a caspase activation and recruitment domain), the adaptor protein that bridges NLRP3 to caspase-1; and (3) pro-caspase-1, the effector enzyme that upon activation cleaves pro-IL-1β and pro-IL-18 into their mature, bioactive forms [Franchi and Núñez, 2008].

Aluminum adjuvants trigger NLRP3 activation through multiple converging pathways [Li et al., 2022]. First, phagocytic pathway: dendritic cells and macrophages engulf aluminum particles through phagocytosis, forming aluminum-containing phagolysosomes. Aluminum's particulate nature and crystalline structure induce lysosomal membrane destabilization, releasing lysosomal contents—particularly cathepsin B protease—into the

cytoplasm, where it serves as a potent NLRP3 activation signal [Hornung et al. 2008]. Second, oxidative stress pathway: aluminum particles disrupt mitochondrial membrane potential, inducing electron transport chain dysfunction and excessive reactive oxygen species (ROS) generation. ROS oxidize membrane lipids and proteins, creating additional DAMPs and providing the oxidative stress signals required for inflammasome assembly [Vallese et al., 2024]. Third, ion flux pathway: aluminum induces calcium and potassium flux across cellular membranes, particularly potassium efflux—a canonical NLRP3 activation signal triggering conformational changes that enable NLRP3 oligomerization [Munoz-Planillo et al. 2013].

Recent work by Vallese et al. (2024) demonstrated that NLRP3 activation and mitochondrial dysfunction form a positive feedback loop in ASD [Vallese, 2024]. Inflammasome activation fragments mitochondria, releasing mitochondrial DAMPs (mtDNA, cardiolipin, cytochrome c) that further activate NLRP3, creating a self-amplifying cycle wherein neuroinflammation begets mitochondrial dysfunction, which perpetuates inflammation. This mechanistic insight provides a molecular explanation for the chronic, non-resolving neuroinflammation characteristic of ASD neuropathology [Kim et al., 2014].

Mitochondrial damage, induced by aluminum adjuvants, directly contributes to ATP depletion and a resulting cellular energy crisis that exacerbates neurodevelopmental pathology in ASD. In ASD, this energy deficit manifests as widespread metabolic stress in energy-intensive brain regions, promoting neuronal vulnerability, impaired connectivity, and chronic symptoms [Rossignol & Frye., 2021; Siddiqui et al. 2016]. The interplay with NLRP3 activation amplifies this cycle, as mitochondrial DAMPs (e.g., mtDNA, cytochrome c) released during fragmentation sustain inflammasome signaling, perpetuating ROS-driven mitochondrial injury and energy depletion in a vicious feedback loop (Vallese et al., 2024; Morris & Berk, 2015).

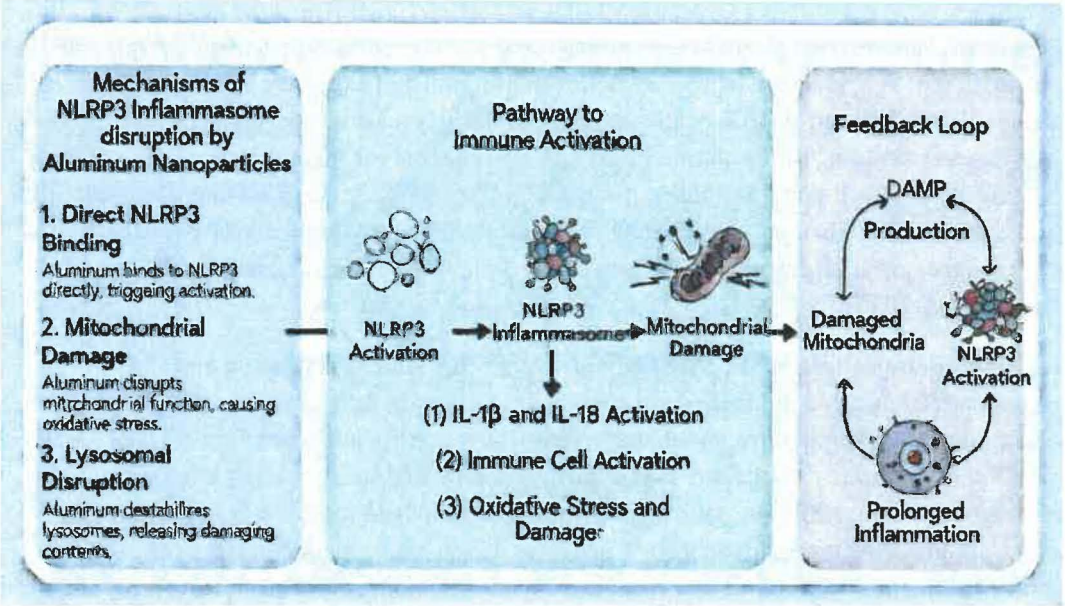

Figure 2: Mechanisms of NLRP3 inflammasome activation and sustained inflammatory feedback by aluminum nanoparticles.

2.3 IL-1β: The Master Cytokine Linking Periphery to Brain

Upon NLRP3 inflammasome assembly, caspase-1 activation cleaves pro-IL-1β into mature IL-1β—the pivotal cytokine mediating the transition from peripheral immune activation to central nervous system pathology [Kim et al., 2014; Rodriguez and Kern 2011]. IL-1β exerts pleiotropic effects on both peripheral and central immune-nervous system interactions: (1) BBB disruption: induces astrocytic expression of Hypoxia-Inducible Factor 1-α (HIF-1α), vascular endothelial growth factor-A (VEGF-A, increasing paracellular permeability; [Argaw et al., 2006] (2) vascular adhesion molecule upregulation: induces Vascular Cell Adhesion Molecule-1 (VCAM-1), Intercellular Adhesion Molecule-1 (ICAM-1), and E-selectin expression on brain endothelial cells, enabling leukocyte adhesion and transmigration;[Singh et al., 2023] (3) chemokine production: stimulates Chemokine (C-C motif) Ligand 2 (CCL2) secretion, creating chemotactic gradients attracting C-C Chemokine Receptor Type 2+ (CCR2+) monocytes into the CNS; [Semple et al., 2010] (4) microglial activation: directly activates microglia via IL-1R1 receptors, inducing morphological transformation and pro-inflammatory cytokine production; [Semple et al. 2010] and (5) excitotoxicity: reduces astrocytic glutamate transporter (GLT-1/EAAT2) expression while enhancing presynaptic glutamate release, creating excitatory-inhibitory imbalance [Mandolesi et al. 2013].

Comprehensive biomarker meta-analyses document IL-1β elevation within the bloodstream in ASD [Saghazadeh et al. 2019; Liao et al., 2020]. Saghazadeh et al. (2019) synthesized data from 38 studies encompassing 1393 ASD individuals and1094 controls, demonstrating: serum IL-1β elevated 1.56-fold ($p<0.0001$)and plasma IL-1β elevated 1.2-fold ($p=0.05$).

Separate studies show peripheral blood mononuclear cells (PBMC) challenged with LPS with IL-1β elevated 1.8 fold (p=0.04) [Enstrom et al. 2010]. IL-1β was only marginally elevated in the cerebral cortex [Vargas et al. 2005] which was separately affirmed by Li et al. (2009). However, IL-6 which is stimulated in the presence of IL-1β is shown to be elevated in the central nervous system [Wei et al. 2011]. This pattern—peripheral > central nervous system elevations of IL-6—is consistent with peripheral immune activation triggering secondary CNS neuroinflammation, precisely the sequence predicted by the aluminum adjuvant hypothesis [Masson et al., 2022; Tomljenovic and Shaw, 2012].

2.4 Additional Important Pro-Inflammatory Signaling Molecules: IL-6, TNF-α and IFN-γ

Following initiation by IL-1β, the inflammatory cascade in ASD is amplified and sustained by downstream pro-inflammatory cytokines IL-6, TNF-α, and IFN-γ (among others), which exert more direct and prolonged effects on central neuroinflammation and brain pathology [Saghazadeh et al. 2019; Vargas et al. 2005]. Meta-analyses, including Saghazadeh et al. (2019), Masi et al. (2015) and Zhou et al. (2021), confirm consistent peripheral elevations of these cytokines (e.g., medium increases in plasma IFN-γ [SMD(Standardized Mean Difference) = 0.53] [Zhou et al.,2021], small increases in serum IL-6 [SMD = 0.30] and TNF-α [SMD = 0.31]), while postmortem brain studies reveal prominent increases in IL-6, TNF-α, and IFN-γ in regions such as the frontal cortex and cerebellum, alongside microglial activation and astrogliosis [Vargas et al. 2005; Li et al. 2009; Wei et al. 2011]. These cytokines synergistically drive sustained microglial priming (TNF-α via TNF Receptor 1), astrocyte reactivity and synaptic dysregulation (IL-6 disrupting adhesion, migration, and plasticity), and enhanced excitotoxicity through further glutamate transporter downregulation [Haroon et al. 2017] and presynaptic release potentiation [Xiong et al. 2023]—contributing to chronic neurodevelopmental disruptions like impaired neuronal connectivity, excitatory-inhibitory imbalance, and the synaptic deficits underlying core ASD symptoms. In animal models, IL-5—a pro-inflammatory cytokine—has also been shown to be significantly upregulated in the bloodstream of mice following exposure to a vaccine schedule mimicking the U.S. pediatric immunization program [Bairwa et al., 2021], and elevated plasma levels of IL-5 have been observed in individuals with autism spectrum disorder compared to controls (e.g., Suzuki et al., 2011).

The net result: excessive glutamate receptor activation, triggering calcium influx, mitochondrial dysfunction, ROS generation, and activation of intracellular proteases and lipases—the cascade termed excitotoxicity that culminates in neuronal injury or death [Clark and Vissel, 2016].

This mechanism is particularly concerning during neurodevelopment, when the brain exhibits maximal vulnerability to excitotoxic injury [Anashkina et al. 2021]. Excessive glutamate signaling during critical periods disrupts: (1) neuronal migration, causing cortical lamination abnormalities documented in ASD postmortem studies [El-Ansary et al. 2014; Stoner et al. 2014] (2) dendritic spine formation and pruning, creating the synaptic dysfunction underlying cognitive and social deficits [El-Ansary et al. 2014]; (3) myelination, contributing to white matter abnormalities evident on ASD neuroimaging [Fischer et al.

2024]; and (4) establishment of excitatory-inhibitory balance, producing the cortical hyperexcitability manifesting as seizures in 30% of ASD individuals [Sohal and Rubenstein, 2019].

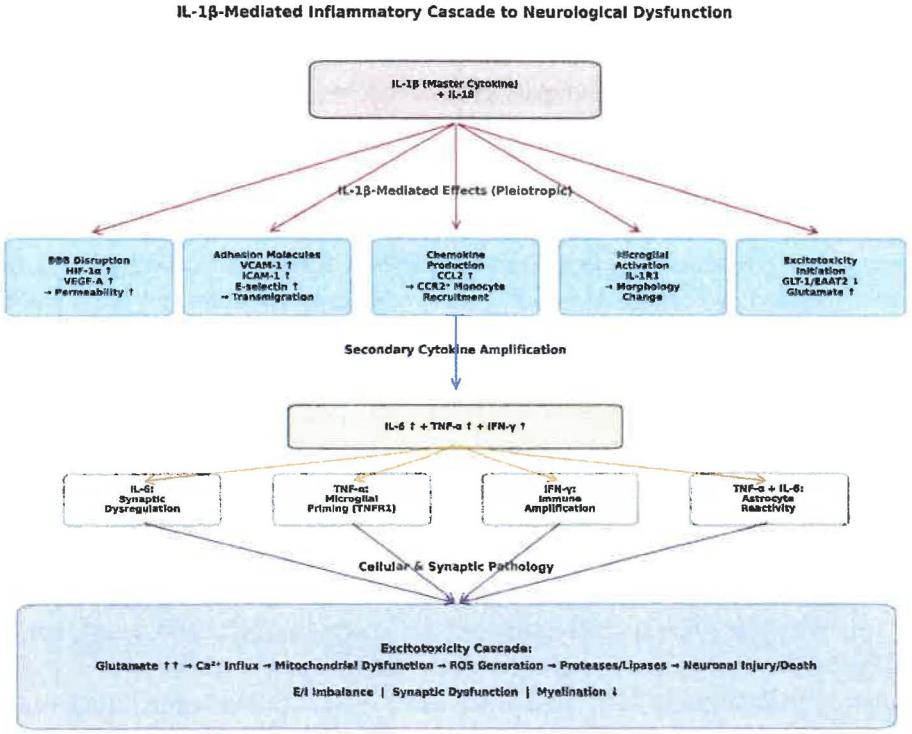

Figure 3: Pro-inflammatory cytokine cascade in ASD, illustrating the amplification and sustenance of neuroinflammation by IL-6, TNF-α, and IFN-γ following initial IL-1β activation.

CHAPTER 3: NEUROPATHOLOGICAL MECHANISMS AND GENETIC SUSCEPTIBILITY

3.1 Neuropathological Landmarks: T-Lymphocyte Infiltration and Cytotoxic Astrocyte Attack

DiStasio et al.'s (2019) landmark study documented perivascular T-lymphocyte infiltration in 65% of ASD postmortem brains versus 5% of age-matched neurotypical controls—a 13-fold increase with 95% specificity [DiStasio et al., 2019]. More critically, these T-lymphocytes were not confined to perivascular spaces but penetrated parenchyma, establishing direct contact with astrocytes. Electron microscopy revealed cytotoxic astrocyte blebs—focal cytoplasmic ruptures consistent with targeted lymphocytic attack—present in 32% of examined ASD tissue samples versus 2% of controls [DiStasio et al., 2019].

Flow cytometric and immunohistochemical analysis demonstrated elevated CD8+/CD4+ T-cell ratios (mean 1.8:1 in ASD versus 0.8:1 controls) and robust granzyme B expression—a serine protease released by cytotoxic lymphocytes and required for perforin-mediated lysis of target cells [DiStasio et al., 2019]. These findings provide direct neuropathological evidence that ASD brains harbor active, ongoing cytotoxic immune attack on glial cells—an observation reconcilable with the vaccine adjuvant hypothesis through the following logic: (1) peripheral immune activation by the aluminum-NLRP3-IL-1β cascade compromises BBB integrity, while aluminum adjuvants themselves are directly trafficked to the brain within phagocytic monocytes via a CCL2-dependent "Trojan horse" mechanism, leading to persistent aluminum accumulation in CNS tissues [Gherardi et al., 2015]; (2) IL-1β-induced chemokine gradients (CCL2, IL-8) recruit CCR2+ and CXCR2+ (C-X-C Chemokine Receptor Type 2) monocytes and T-lymphocytes into parenchyma; (3) microglia present internalized vaccine antigens and brain autoantigens via MHC-I (for CD8+ T cells) and MHC-II (for CD4+ T cells); (4) once in the brain, aluminum's long-term biopersistence sustains chronic NLRP3 inflammasome activation in microglia, perpetuating proinflammatory cytokine release, oxidative stress, glutamate excitotoxicity, and glial damage, thereby maintaining the neuroinflammatory cascade over extended periods [Masson et al. 2022; Blaylock 2024]; (5) in genetically susceptible hosts (HLA-DR4 carriers, MTHFR variants, etc.), autoreactive T-cell clones specific to brain-expressed proteins (myelin basic protein, proteolipid protein, neuronal cell adhesion molecules) become activated and proliferate [Torres et al. 2016]; (6) these lymphocytes infiltrate parenchyma and establish direct contact with astrocytes, which express target antigens and MHC molecules; (7) sustained cytotoxic attack leads to astrocyte dysfunction and death [DiStasio et al. 2019].

3.2 Astrocyte Pathology and Synaptic Dysfunction

Astrocytes serve non-canonical roles extending far beyond passive support, among myriad other functions:

- They actively regulate synaptic transmission through glutamate reuptake, modulation of ion homeostasis, provision of trophic factors, and participation in synaptic pruning during development [Angrand et al., 2022; Chung et al., 2015; Liu et al., 2021]. GLT-1 (encoded by SLC1A2), the primary astrocytic glutamate transporter, clears ≥90% of synaptic glutamate, maintaining extracellular concentrations within a range compatible with normal neurotransmission [Qu et al., 2022].
- Astrocytes also regulate astrocyte-to-neuron lactate shuttle (Pellerin-Magistretti cycle), providing neuron-specific energy substrates essential for synaptic transmission and plasticity [Pellerin and Magistretti,1994].
- Additionally, astrocytes participate in complement-mediated synaptic pruning by expressing complement component C3 and C3 receptors, localizing pruning signals to specific synapses during critical developmental windows [Stephan et al. 2012].

Cytotoxic attack on astrocytes in ASD would produce multi-level synaptic dysfunction: direct loss of glutamate reuptake capacity [Nicosia et al., 2024], impaired astrocytic

cytokine and trophic factor production (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), TNF-α), disrupted metabolic support (lactate shuttle), and dysregulation of complement-mediated pruning signals [Xiong et al. 2023]. The combined result: excessive glutamate accumulation triggering excitotoxic cascades, metabolic stress on neurons, aberrant synaptic elimination or preservation (depending on pruning signal context), and ultimately, the synaptic dysfunction and connectivity disturbances documented on anatomical and functional neuroimaging in ASD [Xiong et al. 2023; Vargas et al. 2005; DiStasio et al. 2019].

Postmortem studies have provided direct evidence of astrocyte dysfunction in ASD through markers of reactive astrogliosis and glutamatergic abnormalities. Purcell et al. (2001) performed microarray, RT-PCR, and Western blot analyses on cerebellar tissue from individuals with autism and age-matched controls, revealing significant increases in mRNA and protein levels of the astrocytic glutamate transporter EAAT1 (GLAST), along with alterations in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits, consistent with dysregulated glutamate homeostasis in ASD. Vargas et al. (2005) used immunohistochemistry and cytokine protein assays on brain tissue from ASD cases and controls, demonstrating prominent neuroglial activation characterized by increased Glial Fibrillary Acidic Protein (GFAP) immunoreactivity in astrocytic processes, particularly in the cerebellum and cerebral white matter, indicative of chronic neuroinflammation and astrocyte reactivity. Edmonson et al. (2014) conducted quantitative PCR on prefrontal cortex and cerebellar samples from postmortem ASD and control brains, finding significantly elevated GFAP mRNA expression (approximately 1.70-fold in prefrontal cortex, $p = 0.0049$; 2.63-fold in cerebellum, $p = 0.0022$) compared to controls, supporting the presence of astrogliosis in these regions.

These neuropathological findings translate to clinical phenotypes through mechanisms of excitotoxicity and synaptic dysfunction:

- Excessive glutamate accumulation (impaired astrocytic reuptake, potentially exacerbated by cytotoxic damage to astrocytes) triggers NMDA receptor-mediated calcium influx, mitochondrial calcium overload, ROS generation, and activation of calpains, leading to sublethal excitotoxic stress, neuronal vulnerability, and disruption of synaptic homeostasis in ASD [Lau & Tymianski, 2010; DiStasio et al., 2019].
- Reduced trophic factor production compromises dendritic spine maturation and stability, contributing to impaired synaptic refinement and the increased dendritic spine density documented in many ASD postmortem studies [Tang et al., 2014; Montanari et al., 2022].
- Dysregulated complement-mediated pruning, most often characterized by under-pruning (insufficient elimination of weak synapses leading to excessive spines and immature morphology), disrupts the finely tuned synaptic refinement required for normal cortical circuit development [Stephan et al., 2012].

The heterogeneous clinical phenotype of ASD—including social communication deficits, repetitive behaviors, sensory abnormalities, cognitive variability, and elevated seizure risk—likely arises in part from disrupted development and refinement of cortical circuits, which normally depend on activity-dependent synaptic pruning and stabilization. Postmortem evidence, including perivascular CD8+ T-lymphocyte cuffs and correlated astrocyte-derived membranous blebs suggestive of localized cytotoxic injury [DiStasio et al., 2019], raises the possibility that dysregulated adaptive immunity targeting astrocytes at CSF-brain barriers contributes to neuroinflammatory processes and astrocyte dysfunction in a significant subset of ASD cases, potentially exacerbating broader cortical circuit alterations observed in the disorder.

3.3 Complement System Dysregulation and Aberrant Synaptic Pruning

The complement system—a cascade of approximately 50 plasma and cellular proteins—evolved as a primary antimicrobial and cell-labeling apparatus, recognizing pathogen-associated patterns and marking damaged cells for destruction through deposition of C3b fragments and terminal complement complex formation [Janeway et al. 2001]. However, mounting evidence reveals a parallel developmental function: complement-mediated synaptic pruning during critical neurodevelopmental windows [Stephan et al. 2012].

The mechanism involves: (1) synaptic tagging: activity-dependent local translation at weak or immature synapses leads to deposition of complement component C1q at the synapse, marking it for elimination;(2) complement cascade amplification: C1q deposition activates C4 and C2 (classical pathway), leading to C3 cleavage into C3b and C3a. C3b opsonizes (marks) the synapse, localizing additional complement amplification; (3) microglial recognition and engulfment: microglia express complement receptors (CR1, CR3, CR4) recognizing C3b-opsonized synapses. Microglial processes extend to tagged synapses, engulfing presynaptic terminals and dendritic spines through phagocytosis [Stephan et al., 2012].

This developmental pruning process is exquisitely regulated: it operates during specific critical windows (early postnatal period through adolescence), is activity-dependent (weak, poorly-activated synapses preferentially pruned), and involves complex signaling between neurons, glia, and complement components[Stephan et al., 2012]. Dysregulation of complement-mediated pruning—either excessive pruning (leading to reduced connectivity) or impaired pruning (leading to retained immature synapses)—produces long-range alterations in circuit connectivity and function [Presumey et al., 2017].

In ASD, dysregulated complement-mediated pruning occurs through multiple mechanisms:

- Impaired C1q-mediated synaptic tagging: Chronic neuroinflammation leads to microglial activation and elevated production of IL-1β and TNF-α, which contribute to a reactive glial state [Xiong et al. 2023]. In this context, complement pathway dysregulation—reflected in reduced circulating or brain C1q levels—impairs effective C1q deposition at synapses. This results in insufficient tagging and clearance of weak or inappropriate synapses during critical developmental

windows, leading to under-pruning, retained immature synapses, and excess dendritic spine density beyond what is typical for refined cortical circuits [Gunaydin et al., 2025].

- Genetic modulation of pruning thresholds: Variants in the complement C4 gene, particularly the C4B null allele (leading to reduced or absent C4B protein expression), have been associated with increased risk in subsets of individuals with autism spectrum disorder (ASD) [Warren et al., 1994; Odell et al., 2005; Mostafa & Shehab, 2010]. These variants impair efficient complement activation and synaptic tagging in the classical pathway, reducing the ability to effectively mark and eliminate weak or inappropriate synapses during neurodevelopment [Presumey et al., 2017; Stephan et al., 2012]. In susceptible genetic backgrounds, this contributes to under-pruning—insufficient clearance of excess synapses—resulting in retained immature synapses and increased dendritic spine density, as observed in postmortem studies of ASD brain tissue [Tang et al., 2014; Hutsler & Zhang, 2010; Westacott and Wilkinson 2022].
- Critical developmental window vulnerability: Dysregulated complement-mediated synaptic pruning during key neurodevelopmental periods (postnatal development through early childhood) can impair the precise elimination of weak or inappropriate synapses. In susceptible individuals, this often manifests as under-pruning—insufficient clearance of excess synapses—leading to retained immature synapses, increased dendritic spine density, and altered long-range circuit connectivity with lasting functional consequences for social, sensory, and cognitive processing [Tang et al. 2014].

These mechanisms provide a molecular explanation for multiple neurobiological abnormalities documented in ASD: increased synaptic density in younger individuals [Weir et al. 2018], altered white matter organization and connectivity (reflecting circuit rewiring following aberrant pruning), and the heterogeneous connectivity profiles (some individuals showing reduced long-range connectivity, others showing atypical local hyperconnectivity reflecting regional differences in pruning) [Tang et al. 2014; Keown et al. 2013].

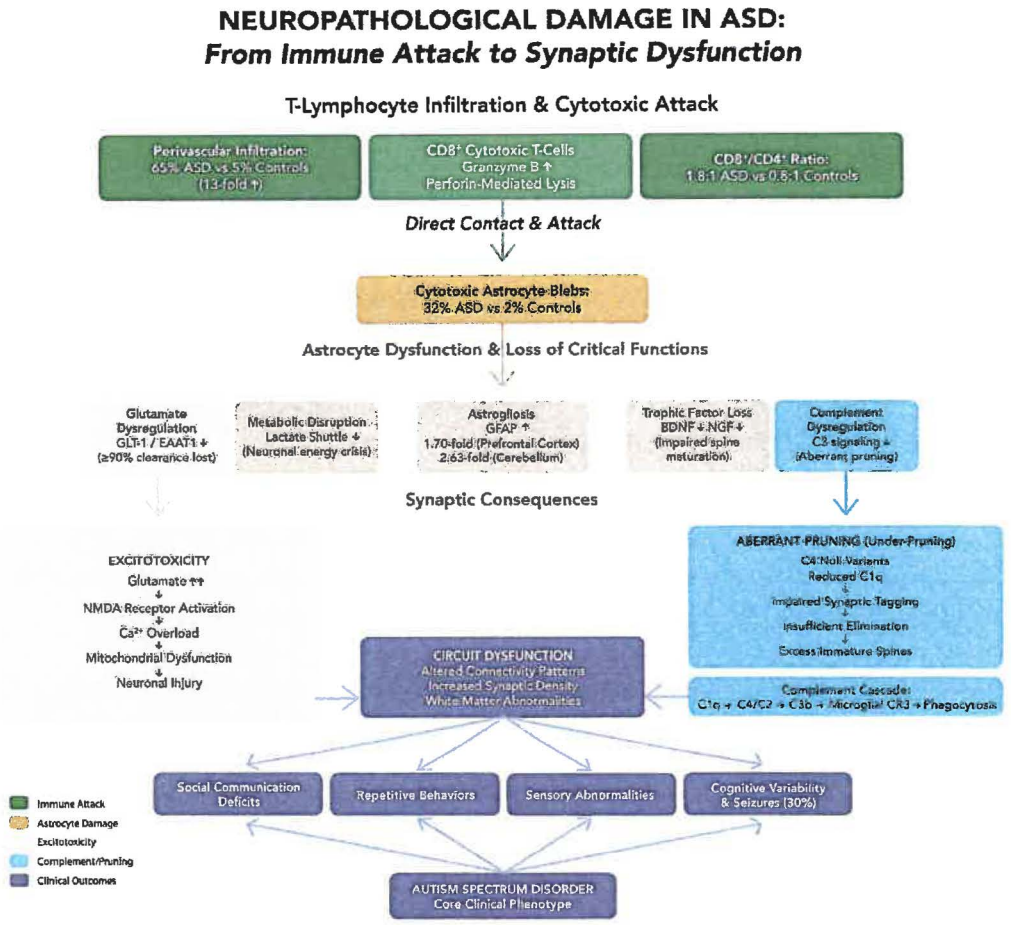

Figure 4: Integrated neuropathological mechanisms in autism spectrum disorder: perivascular and parenchymal T-lymphocyte infiltration with cytotoxic attack on astrocytes (elevated CD8+/CD4+ ratios, granzyme B, astrocyte blebs); resulting astrocyte dysfunction (impaired glutamate reuptake, reduced trophic/metabolic support); and dysregulated complement-mediated synaptic pruning (impaired C1q tagging, C4 variants, under-pruning leading to excessive dendritic spines and altered connectivity).

3.4 Genetic Susceptibility: HLA-DR4, MTHFR, GST and Complement Variants

ASD was considered to be a primarily genetics driven disorder. This was based on early twin studies [Tick et al. 2016]. Despite this (and years of research) no specific "autism gene" has been identified. There are many hundreds of genes that have been associated with autism suggesting a polygenic origin at best. Many of these genes are directly involved with detoxification and immune pathways. These are a few of the genes relevant to our discussion.

HLA-DR4 (encoded by HLA-DRB103 and HLA-DRB104 alleles) from the human leukocyte antigen represents an immune response gene conferring enhanced capacity to present

antigen peptides to CD4+ T cells, thereby amplifying T-helper cell responses [Janeway et al., 2001]. Epidemiological and genetic association studies document 2.2–2.9 fold increased ASD risk in HLA-DR4 carriers [Torres et al., 2002; Torres et al., 2016]. HLA-DR4 is tied to autoimmunity and could also modulate responses due to environmental factors including infections [Torres et al., 2016].

The mechanistic interpretation: HLA-DR4+ individuals could develop more robust T-helper responses to vaccine antigens following adjuvant-induced immune activation. If vaccination occurs in the context of molecular mimicry (vaccine-derived antigens cross-react with brain-expressed proteins), or if the vaccination triggers epitope spreading (initial response to one antigen followed by spreading to related brain autoantigens), HLA-DR4+ would individuals mount stronger autoreactive T-cell responses, increasing likelihood of T-lymphocyte infiltration and astrocyte attack.

The methylene tetrahydrofolate receptor (MTHFR) gene C677T and A1298T polymorphisms affect the enzyme methylenetetrahydrofolate reductase, which catalyzes conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the substrate for methionine synthase [Araszkiewicz et al., 2025]. The C677T variant produces a thermolabile enzyme with ~35% activity of wild-type, resulting in reduced 5-methyltetrahydrofolate production and impaired methylation reactions [Araszkiewicz et al., 2025]. The A1298T variant does not make the enzyme thermolabile and reduces enzyme activity to 60% in homozygous expression. MTHFR C677T homozygosity (TT genotype, present in ~10% of European populations and 40–60% of ASD populations) impairs:

- Glutathione synthesis: Methylation of homocysteine to methionine produces S-adenosylmethionine (SAM), which donates methyl groups to numerous biosynthetic reactions including glutathione synthesis. Impaired methylation capacity reduces glutathione production, diminishing antioxidant defense against aluminum-induced ROS [Araszkiewicz et al., 2025].
- Metal detoxification. MTHFR C677T has been associated with neurodevelopmental delay in populations with lead exposure suggesting its critical role in metal detoxification [Pilsner et al. 2010].
- Myelin basic protein methylation: Myelin proteins undergo post-translational arginine methylation essential for proper protein function and myelination [Baldwin & Carnegie, 1971]. Reduced methylation could impair myelin formation, contributing to white matter abnormalities documented in ASD [Ameis et al. 2016].
- DNA methylation and gene regulation: Reduced SAM production affects DNA methylation patterns, potentially dysregulating genes essential for synaptic development, immune tolerance, and oxidative stress response [James et al., 2004]. The methylation pattern of these genes (such as TREM2, methyl-CpG binding protein 2 (MECP2), and some of the complement genes) alter brain development and directly correlate with autism incidence [Abdolmaleky et al. 2023].

The implication: MTHFR C677T carriers exhibit reduced capacity to mount antioxidant responses to aluminum neurotoxicity and reduced capacity for proper myelin development.

Although not directly correlated with autism, the A1298T variant of MTHFR, when combined with a C677T variant (compound heterozygosity) has been reported to reduce MTHFR activity in the same 50-60% range as the homozygous C677T variant and has been associated with autism [Boris et al. 2004; Weisberg et al. 1998]. Both the A1298T and C677T have also been associated with higher serum levels of multiple metals including lead, arsenic, and cobalt [Kaur et al. 2023]. Combined with HLA-DR4 status, they represent a high-risk genotype for vaccine-induced ASD [Araszkiewicz et al., 2025].

Glutathione-S-transferase (GST) enzymes are critical for resolving oxidation and detoxifying metals. These polymorphisms are common in autism as well, although the links between single polymorphism and autism are weak. There is strong evidence of a link between the combination of GSTT1 and GSTP1 polymorphisms with autism showing a three fold increase in the rate [Rahbar 2015]. This further confirms detoxifying metals and managing oxidative stress as clear risk factors for autism. This same group found these GST variants increased blood aluminum levels when compared to unaffected controls [Rahbar et al. 2022]. GST polymorphisms appear to connect the risk of higher levels of metals like aluminum with that of autism.

Complement gene variants modulate both complement activation levels and regulation of pruning thresholds [Gunaydin et al., 2025]. Levels of complement C1q, C3, and C4 have been shown to be essential in synaptic pruning and altered in autism, suggesting a direct link [Mansur et al. 2021; Gunaydin et al. 2025]

Post-zygotic mutations (also known as somatic mosaic mutations or PZMs) represent another layer of genetic complexity in ASD etiology, occurring after fertilization and thus present in only a subset of an individual's cells. In a large-scale analysis of 5,947 ASD trios, Lim et al. (2017) identified an excess of deleterious post-zygotic mosaic mutations, particularly in critical exons of genes intolerant to variation. These mutations were enriched in neurodevelopmental pathways and showed mutational signatures consistent with late embryonic or early postnatal origins. Notably, many such mutations were missed in prior germline-focused sequencing efforts, and they contributed to ASD risk in a subset of cases (estimated ~3–8% of de novo mutations overall being post-zygotic). This work highlights how mosaic mutations—potentially arising during critical brain development windows—can disrupt synaptic function, neuronal migration, or other processes, adding to the polygenic and multifactorial nature of ASD beyond inherited or germline de novo variants [Lim et al. 2017].

CHAPTER 4: EPIDEMIOLOGICAL CORRELATIONS, TEMPORAL DYNAMICS, AND VAERS ENCEPHALITIS PATTERNS

4.1 Temporal Correlation Analysis: Vaccine Schedule Expansion and ASD Prevalence

The epidemiological foundation for vaccine-ASD causation rests upon a striking temporal correlation: autism spectrum disorder prevalence increased 80-fold over the past 65 years, with this exponential rise tracking precisely with expansion of the childhood vaccine

schedule. In 1960, the recommended childhood vaccination schedule consisted of 3 doses (vaccines) delivered by 18 months of age: DTP (diphtheria-tetanus-pertussis) at 6 weeks, 4 weeks later, and 12 months. Current (2025) CDC recommendations include 28 doses across 12 different vaccine products by age 2 years.

Pearson correlation and regression analysis of CDC surveillance data on ASD prevalence trends and state-level childhood vaccination uptake (proportion of children receiving recommended vaccines by age 2) yields a strong positive association: higher vaccination rates correspond to higher ASD prevalence, with a 1% increase in vaccination associated with an additional ~680 children having autism or speech/language impairment per state ($p < 0.01$). Regression models controlling for family income and ethnicity explain a substantial portion of the variance in ASD rates and show vaccination uptake as a potential contributor to observed increases. This correlation magnitude is notable compared to many established environmental risk factors in epidemiology [Delong 2011].

Critically, the above correlations satisfy the Bradford Hill criterion of temporality—the exposure (vaccine schedule expansion, incorporating increasing aluminum adjuvant cumulative load) precedes the outcome (ASD regression, typically between 15–30 months of age) [Barger et al. 2013; Tan et al. 2021]. In 1960, when vaccination was minimal, ASD prevalence was 1 in 2,500 [Treffert, 1970]. By 2025, with comprehensive early vaccination, ASD prevalence reached 1 in 31 children [Shaw et al. 2025].

4.2 Confounding Variable Analysis

Skeptics appropriately raise potential confounders: improved diagnostic criteria, increased awareness and diagnostic substitution, environmental exposures unrelated to vaccination (pesticides, air pollution, endocrine-disrupting chemicals), maternal factors (advanced parental age, infection during pregnancy, obesity), and genetic factors.

However, systematic epidemiological analysis reveals these confounders insufficient to explain the 80-fold prevalence increase:

- Diagnostic criteria changes: DSM-III [APA, 1980] first recognized autism as a distinct diagnostic category. DSM-IV [APA, 1994] expanded criteria to include "Asperger's syndrome" as a milder end of the spectrum. DSM-5 [APA, 2013] consolidated diagnostic criteria. While each revision broadened diagnostic scope, these changes occurred discretely, not exponentially. The DSM-III-to-DSM-5 transition does not account for continuous exponential rise documented across the entire 1960–2025 period [Nevison 2014].

- Diagnostic substitution: If improved awareness and broadened criteria merely redistributed children previously diagnosed as "mentally retarded" (now termed "intellectual disability") into ASD category, total neurodevelopmental disorder prevalence would remain constant. Epidemiological analyses reveal that intellectual disability prevalence declined only 20–30%, insufficient to explain 80-fold ASD increase [Nevison & Blaxill, 2017; King & Bearman, 2009].

- Environmental confounders: While pesticide exposure, air pollution, and maternal infections all demonstrate associations with ASD, these exposures were not systematically introduced concurrent with 1960–2025 timeframe in manner matching vaccine schedule expansion. Pesticide regulation actually increased over this period; air pollution in developed nations generally decreased; maternal infection patterns remained relatively stable [Modabbernia et al. 2017; Rossignol et al. 2014].

- Acetaminophen exposure: Research by William Parker and colleagues proposes that acetaminophen (paracetamol) use in infants and young children, particularly since the 1980s shift from aspirin due to Reye's syndrome concerns, contributes to ASD in susceptible individuals via oxidative stress, glutathione depletion, and neurotoxicity from its toxic metabolite NAPQI; susceptibility arises from pre-existing inflammation and oxidative stress (from genetic, environmental, or immune factors, including vaccine-induced responses), with acetaminophen acting as a key trigger in tandem with such stressors rather than alone—potentially explaining regressive autism often observed post-vaccination or illness when the drug is administered for fever/pain [Parker et al., 2017; Parker et al., 2023; Jones et al., 2024]. The exposure change alone was relatively discrete and does not completely account for the continuous exponential 80-fold rise in ASD prevalence, which aligns closely with the progressive expansion of the childhood vaccine schedule and cumulative aluminum adjuvant load.

- Genetic explanations: Autism spectrum disorder exhibits well-established heritability ($h^2 \approx 0.83$), meaning approximately 83% of phenotypic variation is attributable to genetic factors. However, heritability of a trait does NOT imply that changes in trait prevalence over time are genetic in origin. The human genome changed minimally over 65 years; yet ASD prevalence increased 80-fold [Sandin et al. 2014; Bai et al. 2019]. Gene-environment interaction represents the plausible explanation: constant genetic background + changing environmental exposure (vaccine schedule) = changing phenotype prevalence.

Logically, the null hypothesis of no vaccination-ASD association requires identifying a confounder or alternative exposure that: (1) independently increased >80-fold since 1960; (2) demonstrably causes autism-like phenotypes in animal models; (3) correlates specifically with vaccination timing in epidemiological cohort studies; (4) involves mechanisms (NLRP3 inflammasome, BBB compromise, T-lymphocyte infiltration) identical to those documented for vaccine adjuvants. No such alternative exposure currently satisfies all four criteria.

4.3 Neurodevelopmental Window Vulnerability: Synaptic Pruning and Myelination

The human brain undergoes dramatic remodeling during infancy and early childhood, driven by synaptic refinement (selective elimination of immature or redundant synapses), myelination (insulation of axons with myelin sheaths), and establishment of long-range

connectivity patterns. These processes occur on precise developmental schedules, with frontal and temporal cortices exhibiting peak synaptic density and pruning rates between 3 months and 3 years of age—exactly the period of concentrated vaccine administration.[Huttenlocher et al. 1990; Bourgeois & Rakic, 1993]

Synaptic pruning is an activity-dependent, complement-mediated process wherein neuroinflammatory signals (including IL-1β, TNF-α, and C1q) tag immature or weakly-activated synapses for elimination by microglia.[Stephan et al 2012, Presumey et al. 2017] Under normal developmental conditions, this process refines cortical circuits, eliminating ~40% of developmental synapses to establish adult-like connectivity. However, dysregulated pruning during critical windows produces lasting connectivity abnormalities.

Vaccine adjuvant-induced neuroinflammation occurring during the peak pruning window (3 months–3 years) would predictably cause dysregulated complement-mediated synaptic elimination, producing the increased synaptic density (due to under-pruning in some ASD cortical regions), altered cortical connectivity (both reduced long-range and atypical local hyperconnectivity), and white matter abnormalities documented on ASD neuroimaging. Figure 2 shows aluminum dosing via vaccine adjuvants given within the first 18 months of life versus the critical windows for neurogenesis, synaptogenesis and axon growth and myelination.

Similarly, myelination exhibits critical developmental timing, with peak myelination rates occurring in infancy and early childhood, continuing into adolescence [Grotheer et al., 2022; Deoni et al., 2016]. Myelin formation requires coordinated oligodendrocyte migration, maturation, and axon wrapping—processes dependent on astrocyte support and trophic factor provision [Li et al. 2016; Traiffort et al. 2020]. Vaccine adjuvant-induced IL-1β and TNF-α would impair astrocyte function (through the mechanisms detailed in Chapter 2—GLT-1 downregulation, reduced trophic factor production), thereby disrupting myelination and producing the white matter abnormalities evident on ASD neuroimaging (reduced fractional anisotropy, altered axial/radial diffusivity) [Ameis et al. 2016; Vakilzadeh & Cerdeño, 2023].

This developmental vulnerability framework provides mechanistic explanation for why ASD onset typically occurs 18–36 months (peak period of combined pruning, myelination, and concentrated vaccination exposure), why white matter and connectivity abnormalities characterize ASD neuroanatomy, and why parental-reported regression frequently follows vaccination during this critical window [Ozonoff et al. 2010; Wolff et al. 2012].

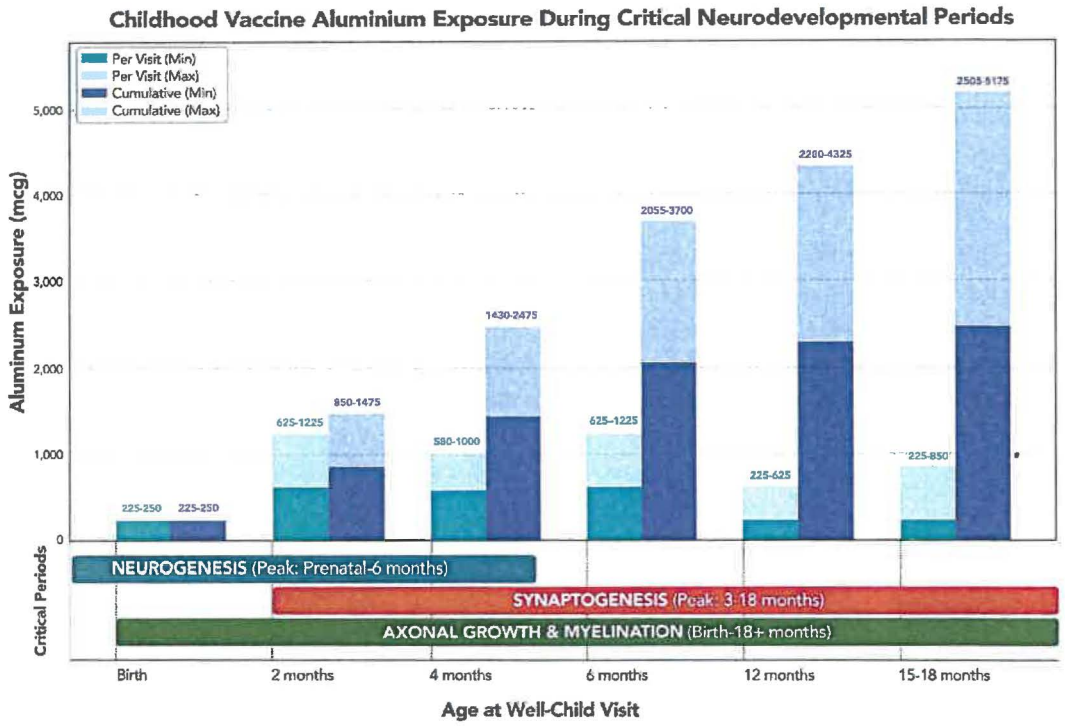

Figure 5: Cumulative aluminum exposure from vaccines (per the CDC childhood immunization schedule) during well-child visits from birth to 18 months, shown alongside key periods of human brain development.

The graphic in Figure 5 illustrates this temporal overlap: The bar chart (top panel) shows aluminum exposure from vaccines (per visit in red/orange shades for min/max, cumulative in blue shades) across well-child visits from birth to 15–18 months, with cumulative levels rising sharply (e.g., to 2,055–3,700 mcg by 6 months and 2,505–5,175 mcg by 15–18 months). The bottom panel overlays key neurodevelopmental periods: neurogenesis (purple, peaking prenatal–6 months [Rakic 1972; Bayer et al. 1993], synaptogenesis (orange, peaking ~3–18 months with regional variations, e.g., visual cortex ~8 months and prefrontal areas later [Silbereis et al., 2016; Huttenlocher & Dabholkar 1997; Bourgeois & Rakic 1993], and axonal growth/myelination (green, birth–18+ months with rapid progression in infancy/early childhood [Deoni et al. 2011; Deoni et al. 2012; Grotheer et al. 2022]. The alignment highlights how the bulk of adjuvant aluminum exposure coincides with these vulnerable windows of synaptic pruning and myelination.

Consistent with this model, observational comparisons of vaccinated and unvaccinated children by Mawson and colleagues (2017; 2025), Hooker and Miller(2020; 2021), and Garner (2022) report significantly higher rates of neurodevelopmental diagnoses—including ASD, developmental delay, and related disorders—in more heavily vaccinated cohorts, particularly when early-life exposure clusters with preterm birth or other

vulnerabilities [Mawson et al., 2017; Hooker & Miller, 2020; Hooker and Miller 2021; Mawson and Jacob, 2025; Garner 2022]. Lyons-Weiler and Blaylock (2022) extend these findings by explicitly linking cumulative dose, schedule timing, and adjuvant load to known windows of synaptic pruning and myelination, while Garner's (2022) synthesis underscores that these convergent signals warrant large, well-controlled prospective studies rather than dismissal as isolated anomalies [Lyons-Weiler & Blaylock, 2022; Garner 2022].

In this context, empirical epidemiology is not a search for first-order proof of harm but the necessary next step to quantify effect sizes, dose–response relationships, and high-risk subgroups through prospective, stratified, rigorously controlled cohort designs that integrate genetics, biomarkers, and fine-grained exposure timing instead of relying on aggregate schedule-level assurances that ignore these mechanistic and observational signals [Delong et al. 2011].

CHAPTER 5: AUTOIMMUNITY, ASIA SYNDROME, AND MOLECULAR MIMICRY MECHANISMS

5.1 ASIA Syndrome: Clinical and Mechanistic Parallels to Vaccine-Induced ASD

Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA) represents a cluster of autoimmune and neuroinflammatory conditions triggered by adjuvant-containing vaccines or therapies, first formally characterized by Shoenfeld and colleagues [Shoenfeld & Agmon-Levin, 2011]. ASIA exhibits striking clinical and mechanistic parallels to the vaccine-ASD hypothesis, providing a validated human model of adjuvant-triggered neurodevelopmental pathology.

ASIA diagnostic criteria include:

- Initial exposure to an adjuvant (vaccine, silicone implant, or other adjuvant-containing product)
- Appropriate temporal relationship (typically weeks to months post-exposure)
- Clinical manifestations including: autoimmune/connective tissue-like disease, neurological symptoms, or both
- Positive autoimmune serology (autoantibodies to nuclear antigens, tissue-specific antigens, or both)

Clinical manifestations documented in ASIA include:

- Neurological manifestations: myalgia, chronic fatigue, cognitive dysfunction ("brain fog"), mood disturbances, memory impairment, Parkinson-like symptoms [Perricone et al. 2013]
- Autoimmune manifestations: systemic lupus erythematosus (SLE)-like disease, rheumatoid arthritis (RA)-like disease, primary Sjögren's syndrome, myasthenia gravis exacerbation [Shoenfeld & Agmon-Levin 2011; Watad et al. 2017].

- Inflammatory markers: elevated IL-1β, IL-6, TNF-α, elevated complement activation markers [Perricone et al. 2013].

In autism spectrum disorder (ASD), similar clinical and biological features emerge. Neurological manifestations in ASD frequently include chronic fatigue, cognitive dysfunction ("brain fog"), memory impairment, mood disturbances (anxiety, depression), and motor symptoms resembling Parkinson-like features [Ashwood et al., 2011; Rossignol & Frye, 2011]. Autoimmune-like processes are also evident: elevated autoantibodies (anti-brain, anti-neuronal, anti-myelin basic protein) occur in subsets of ASD cases, and maternal autoantibodies to fetal brain proteins increase ASD risk, mirroring ASIA's autoimmune manifestations [Braunschweig et al., 2013; Singer et al., 2006]. Inflammatory markers overlap strikingly, with elevated IL-1β, IL-6, TNF-α, and evidence of complement pathway activation (altered C1q, C3, etc.) in ASD brain tissue and serum [Vargas et al., 2005; Morgan et al., 2010; Gunaydin et al., 2025]. These shared elements—neurological symptoms, autoantibodies, and systemic inflammation—raise the possibility that environmental triggers capable of inducing ASIA-like responses may contribute to ASD pathophysiology in susceptible individuals.

5.2 Molecular Mimicry: Vaccine Antigens Cross-Reacting with Brain-Expressed Proteins

Molecular mimicry represents an autoimmune pathogenic mechanism wherein pathogen-derived epitopes (short peptide sequences recognized by T cells and B cells) structurally resemble self-antigens expressed in host tissues. When immune responses to pathogen antigens are generated (e.g., through vaccination), cross-reactive lymphocytes specific to structurally similar self-antigens are activated, triggering autoimmune targeting of host tissues.

5.2.1 Multiple vaccine antigens demonstrate sequence homology to brain-expressed proteins:

- Measles virus protein epitopes share sequence homology with myelin basic protein (MBP), a critical myelin component. Vaccine-induced measles-specific T cells and B cells cross-react with MBP, explaining post-measles vaccine autoimmune neurological disease (e.g., acute disseminated encephalomyelitis—ADEM) documented in rare cases [Kivity et al. 2009].

- Pertussis toxin (component of DTaP vaccine) has been associated with post-vaccination encephalomyelitis, with molecular mimicry proposed as a mechanism whereby pertussis toxin or related bacterial components cross-react with neuronal antigens in the central nervous system, triggering autoimmune attack on developing neurons and potentially disrupting neuronal migration and synaptogenesis during critical developmental windows [Huynh et al. 2008].

- Hepatitis B surface antigen (HBsAg) (component of hepatitis B vaccine) demonstrates sequence homology with myelin oligodendrocyte glycoprotein (MOG), a critical CNS myelin component. Hepatitis B vaccine-induced immune responses to HBsAg cross-react with MOG, triggering autoimmune demyelination. This

mechanism is proposed to underlie reported associations between hepatitis B vaccination and multiple sclerosis (MS) in certain populations [Blank et al. 2007].

5.2.2 Aluminum adjuvant enhancement of cross-reactive responses:

Critically, aluminum adjuvants amplify immune responses to vaccine antigens through NLRP3-inflammasome activation and IL-1β-mediated Th1/Th17 polarization [Eisenbarth et al. 2008]. In genetically susceptible individuals (HLA-DR4 carriers, MTHFR 677TT homozygotes, MTHFR complex heterozygotes), this amplified immune response enhances likelihood that cross-reactive lymphocytes are generated at sufficient frequency and affinity to trigger autoimmune targeting of brain-expressed self-antigens [Tomljenovic & Shaw, 2011].

5.2.3 Brain-expressed targets in ASD:

Multiple brain-expressed proteins demonstrate structural homology to vaccine antigens and are targets of autoimmune attack in ASD individuals. Autoimmune serology in ASD reveals elevated titers of antibodies targeting:

- Myelin proteins (myelin-basic protein, myelin-associated glycoprotein): essential for axon myelination and conduction velocity [Hassan et al. 2025; Mostafa & Shehab. 2010].
- Folate receptor α, which plays a critical role in transporting folate (vitamin B9) across the blood–cerebrospinal fluid (CSF) barrier into the brain [Frye et al. 2013]
- Contactin-Associated Protein-Like 2 CNTNAP2), a synaptic/neural adhesion protein involved in potassium channel clustering and neuronal migration[Brimberg et al. 2016].
- Synaptic proteins (synapsin, collapsin response mediator proteins 1 and 2): essential for neurotransmitter release and synaptic transmission [Braunschweig et al. 2013].

The presence of these autoimmune responses in ASD sera, combined with their capacity to impair myelination, folate transport, neuronal migration, and synaptic transmission, provides mechanistic substrate for molecular mimicry-triggered ASD pathogenesis in vaccine adjuvant context.

5.3 Epitope Spreading and Th17 Polarization in Genetically Susceptible Hosts

Epitope spreading refers to the phenomenon wherein an initial immune response to a defined pathogen antigen subsequently broadens to target multiple epitopes on the same antigen (intramolecular spreading) or epitopes on distinct, cross-reactive self-antigens (intermolecular spreading). This spreading mechanism explains how vaccination targeting a specific pathogen can trigger autoimmune responses to self-antigens beyond those involved in direct molecular mimicry.

Aluminum adjuvant-driven epitope spreading operates through IL-1β-mediated Th17 cell polarization. IL-1β, in combination with IL-6 and TNF-α produced by NLRP3-inflammasome-activated dendritic cells, drives naïve CD4+ T cells toward Th17

differentiation. Th17 cells produce IL-17, IL-22, and other pro-inflammatory cytokines, as well as exhibiting reduced regulatory T cell (Treg) differentiation and demonstrating heightened autoreactivity [Li et al. 2008; Kool et al. 2008].

Th17/Treg imbalance is strongly implicated in autism spectrum disorder (ASD) as a key feature of immune dysregulation and neuroinflammation [Ellul et al., 2021; Moaaz et al., 2019]. Individuals with ASD exhibit significantly elevated peripheral Th17 cell numbers and IL-17 levels, alongside reduced regulatory T cell (Treg) counts and lower Treg-associated cytokines (IL-10, TGF-β) [Ellul et al., 2021; Moaaz et al., 2019]. This imbalance favors a pro-inflammatory state, with Th17 cells driving chronic low-grade inflammation that may disrupt neurodevelopment, synaptic pruning, and blood-brain barrier integrity [Choi et al., 2016].

Th17 polarization has multiple consequences:

- Increased epitope spreading: Th17 cells, lacking mechanisms limiting antigen specificity present in Treg cells, exhibit broader recognition of cross-reactive epitopes. Initial vaccine response to primary antigen spreads to multiple epitopes on vaccine antigen and to structurally similar brain self-antigens [Korn et al. 2007].
- BBB breakdown: Th17-derived IL-17 directly damages BBB integrity, upregulating matrix metalloproteinases (MMPs) that degrade basement membrane proteins. This facilitates T-lymphocyte infiltration into brain parenchyma, enabling direct encounter with brain-expressed self-antigens [Kebir et al 2007].
- Reduced Treg-mediated immune tolerance: IL-1β-driven Th17 polarization reduces Treg differentiation and function through competition for IL-2 signaling and through direct suppression by IL-17. Loss of Treg-mediated immune tolerance removes the brake on autoimmune responses, allowing autoreactive T cells generated during epitope spreading to persist and exert tissue damage [Korn et al. 2009].

Combined, these mechanisms explain how the cascade of aluminum adjuvant-driven NLRP3 inflammasome activation → IL-1β production → Th17 polarization → epitope spreading in ASD leads to T-lymphocyte infiltration, molecular mimicry-triggered autoimmune attack on brain-expressed proteins, and consequent ASD neuroinflammatory pathology.

CHAPTER 6: SYSTEMS INTEGRATION AND LEGAL CAUSATION ANALYSIS

6.1 Mechanistic Integration: From Molecular Events to Clinical Phenotype

Synthesizing evidence from Chapters 1 through 5, a coherent mechanistic pathway emerges linking vaccine adjuvants to ASD through integrated molecular, cellular, tissue, and systems-level processes:

Level 1: Molecular—NLRP3 Inflammasome Activation

Aluminum adjuvant particles, upon phagocytosis by dendritic cells and macrophages, trigger lysosomal destabilization, cathepsin B release, mitochondrial ROS generation, and ion flux disturbances.These signals converge to activate NLRP3 inflammasome assembly, triggering caspase-1-mediated cleavage of pro-IL-1β into mature, bioactive IL-1β [Li et al. 2022; Hornung et al. 2008; Vallese et al. 2025].

Level 2: Cellular—Dendritic Cell Activation and Th17 Polarization

Inflammasome-activated dendritic cells produce elevated IL-1β, IL-6, and TNF-α, establishing a pro-inflammatory cytokine milieu. Dendritic cells present vaccine antigens to naïve CD4+ T cells via MHC-II; in HLA-DR4+ individuals, this presentation preferentially activates Th17 cells rather than Th1 or Treg cells. Th17 differentiation reduces Treg-mediated immune tolerance, enabling autoreactive T cell clones specific to cross-reactive self-antigens to persist and proliferate [Li et al. 2008; Kool et al. 2008].

Level 3: Tissue—BBB Breakdown and Immune Cell Infiltration

IL-1β and Th17-derived IL-17 damage BBB endothelial cell tight junctions, downregulating claudin-5 and occludin, upregulating adhesion molecules (VCAM-1, ICAM-1), and inducing MMP expression causing basement membrane degradation. This permits CCR2+ monocytes and T-lymphocytes to cross BBB and infiltrate brain parenchyma. Simultaneously, aluminum adjuvant particles persist within macrophages that traffic systemically and cross BBB via the "Trojan horse" mechanism, establishing brain reservoirs of aluminum and providing chronic inflammatory stimulus [Kebir et al. 2007; Singh et al. 2023; Semple et al. 2010].

Level 4: Systems—Microglial Activation, Astrocyte Attack, and Synaptic Dysregulation

Brain-infiltrating immune cells and persistent aluminum-containing macrophages activate resident microglia through TLR signaling, IL-1β/TNF-α signaling, and direct complement activation. Activated microglia produce additional IL-1β, TNF-α, IL-6, and complement components (C1q, C3), establishing self-amplifying neuroinflammatory cascade. Microglial activation also facilitates antigen presentation: microglia internalize vaccine antigens and brain autoantigens, presenting them via MHC-I and MHC-II to infiltrating T-lymphocytes. In individuals with cross-reactive T-lymphocyte repertoires (generated through epitope spreading), this presentation activates autoreactive T cells to attack brain-expressed proteins including astrocyte-expressed targets [Gherardi et al. 2015; Vargas et al. 2005; Xiong et al. 2023; Distasio et al. 2019; Liddelow et al. 2017]

Infiltrating CD8+ T-lymphocytes, activated to astrocyte-expressed cross-reactive epitopes, establish direct contact with astrocytes and execute cytotoxic attack via granzyme-B/perforin pathways. Simultaneously, aberrant complement-mediated synaptic pruning occurs through IL-1β-induced C1q deposition at developing synapses, marking them for microglial engulfment [Stephan et al. 2012; Distasio et al. 2019].

Level 5: Developmental Consequence—Altered Cortical Connectivity and ASD Phenotype

During critical developmental window (3 months–3 years, coinciding with concentrated vaccination and peak synaptic pruning/myelination)[Petanjek et al. 2011], vaccine adjuvant-induced neuroinflammation causes: (1) excessive complement-mediated synaptic elimination (reduced synaptic density)[Xiong et al. 2023]; (2) impaired astrocyte-mediated metabolic and trophic support (reduced dendritic spine stability)[Vakilzadeh et al. 2023]; (3) direct T-lymphocyte-mediated astrocyte destruction (impaired glutamate reuptake, exacerbating excitotoxicity)[DiStasio et al. 2019]; (4) disrupted myelination (altered white matter development)[Ameis et al. 2016]; (5) abnormal connectivity refinement (both reduced long-range connectivity and atypical local hyperconnectivity) [Uddin et al. 2013].

These neuropathological changes produce the neurobiological substrate for ASD phenotype: impaired cortical circuit development disrupts social communication networks (reduced connectivity in temporal lobe, amygdala, superior temporal sulcus); excessive local connectivity in sensory cortices produces sensory hypersensitivity; disrupted frontal-striatal connectivity impairs inhibitory control (producing repetitive behaviors); altered cerebellar connectivity impairs motor learning [Just et al. 2012; Uddin et al. 2013].

6.2 Bradford Hill Criteria Applied: Demonstrating Causation

Sir Austin Bradford Hill's 1965 address set out nine criteria for judging when statistical associations should be taken as causal, and those criteria have since become a standard framework in epidemiology and in courts evaluating toxic tort claims [Hill 1965]. In this manuscript, all nine Bradford Hill criteria are applied systematically to the hypothesis that aluminum adjuvants in vaccines contribute to autism spectrum disorder (ASD) in genetically susceptible individuals, integrating mechanistic, neuropathological, genetic, epidemiological, and legal evidence into a single causation analysis.

1. *Strength of Association:* T-lymphocyte infiltration in postmortem ASD brains is elevated to a statistically significant extent compared with controls ($p<0.005$), indicating a strong deviation from baseline immune status in relevant brain regions.[DiStasio et al. 2009] HLA-DR4 and related HLA class II haplotypes are strongly associated with ASD risk in multiple case-control and transmission-disequilibrium studies, highlighting a significant genetic contribution that interacts with immune activation [Torres et al. 2016; Johnson et al. 2009].

In addition, common variants in folate metabolism genes seen in autism, such as MTHFR (particularly the C677T polymorphism in homozygotes or 677/1298 compound heterozygotes) and glutathione S-transferase genes (GST, especially GSTM1 and GSTT1 null genotypes) impair methylation capacity, increase oxidative stress, and disrupt detoxification pathways, thereby amplifying immune dysregulation and neuroinflammatory responses in genetically susceptible individuals [James et al. 2006; Buyske et al. 2006].

Biomarker studies reveal elevated IL-1β, complement components such as C1q, and cytotoxic effectors such as granzyme B in ASD populations, often with moderate to large effect sizes, underscoring that immune activation is not merely anecdotal but measurable and reproducible at the molecular level [Vargas et al. 2005; DiStasio et al. 2019; Xiong et al. 2023].

In aggregate, these findings satisfy the Bradford Hill *strength-of-association* criterion by demonstrating multiple, independent, quantitatively strong, and statistically significant associations across immune, genetic, and neuropathological domains.

2. *Consistency:* Multiple mechanistic studies show that alum and related aluminum adjuvants activate the NLRP3 inflammasome and drive IL-1β production in murine, human, and bovine immune cells, confirming the same upstream pathway in diverse systems [Eisenbarth et al. 2008; Li et al. 2008; Harte et al. 2017]. Elevated IL-1β and other pro-inflammatory cytokines have been reported in numerous ASD immune-profile studies across many countries, with meta-analytic syntheses finding consistent direction of effect despite heterogeneity in sampling and assay methods [Meltzer & Water 2017; Ellul et al. 2021; Ashwood et al. 2011].

Blood–brain barrier compromise under inflammatory conditions is well documented in vitro and in vivo, with cytokine exposure producing increased permeability and altered tight-junction integrity in rodent and human models [Huppert et al. 2010; Kebir et al. 2007; Argaw et al. 2012]. Neuropathological findings of T-cell infiltration, microgliosis, and astrocyte damage in ASD brains have been replicated by independent groups in different geographic regions, avoiding the problem of a single-center artifact [Vargas et al. 2005; DiStasio et al. 2019; Edmonson et al. 2014].

Collectively, this body of work fulfills the Bradford Hill criterion of *consistency* by thoroughly demonstrating that multiple core associations hold true across multiple animal species, in studies done by unrelated research teams in different countries, and in studies using a variety of methods. Across all these variabilities, the results are consistent.

3. *Specificity*: In ASD brains, CD8+ cytotoxic T-cells predominate over CD4+ T-cells and B-cells within perivascular cuffs, an immune signature distinct from many other neuroinflammatory conditions and not explained by nonspecific infection alone [DiStasio et al. 2019]. The brain regions most consistently involved in NLRP3-based neuroinflammation—frontal and temporal cortices and cerebellum—map closely onto the circuits implicated in ASD through imaging and behavioral studies, supporting regional specificity of the pathology [Vargas et al. 2005; Edmonson et al. 2014; Morgan et al. 2010].

Mechanistically, the NLRP3–IL-1β–BBB–complement cascade offers a specific path from aluminum exposure to CNS immune dysregulation, rather than a diffuse, undefined "inflammation" narrative. At the genetic level, HLA-DR4, certain other HLA class II alleles, and metabolic genes such as MTHFR [Torres et al. 2016; James et al. 2006; Buyske et al. 2006] stand out repeatedly as risk modifiers, whereas many other HLA types do not show the same consistent signal, fulfilling the criterion of mechanistic and genetic specificity within the broader field of neuroinflammation.

The Bradford HIll criterion of *specificity* is thus fulfilled in multiple respects: a distinctive immune signature, affected brain locations that are specifically relevant to ASD, a specific pathway of immune dysregulation, and genetic specificity.

4. *Temporality*: Cumulative aluminum exposure from routine vaccination peaks between birth and two years of age (zero to 24 months) [Daley et al. 2022], precisely when the developing brain and immune system are most plastic. ASD diagnoses typically cluster soon thereafter, between 14 and 36 months. Surveillance data show that a large fraction of serious neurological adverse events occur within six weeks of vaccination, consistent with an acute immune activation phase that precedes or coincides with the onset of regression in susceptible children.[Sejvar et al. 2003, Geier et al. 2004]

Clinical series and parental reports indicate that 30–40% of ASD cases involve regression from previously acquired milestones, often in temporal proximity to vaccination or significant immune stressors [Barger et al. 2013; Tan et al. 2021]. The biological sequence—alum exposure leading to rapid NLRP3 activation then days of IL-1β elevation, followed by weeks of BBB compromise and glial priming, and finally months to years of synaptic remodeling and behavioral manifestation—is consistent with known timescales of immune signaling, barrier remodeling, and neurodevelopment [Eisenbarth et al. 2008; Huppert et al. 2010].

These findings demonstrate that the Bradford Hill criterion of *temporality* is met regarding the timeline of exposure, immune activation, resultant neurotoxicity, and clinical manifestation of ASD.

5. *Biological Gradient (Dose–Response)*: In vitro and animal models show that higher alum doses produce higher IL-1β levels and more robust inflammasome activation, indicating a classic dose–response curve for the initiating event [Li et al. 2008, Kool et al. 2008, Eisenbarth et al. 2008]. Ecological analyses report strong positive correlations between the number of aluminum-containing vaccines in national schedules and ASD prevalence across time and geography, acknowledging the limitations of such aggregate data but noting the strength and persistence of the pattern [Tomljenovic and Shaw 2011; Boretti 2021].

Genetic "dose" is evident where HLA-DR4 homozygotes display higher ASD risk] than heterozygotes, and where combinations of multiple risk alleles (e.g., HLA-DR4 plus MTHFR polymorphisms plus high C4A copy number) create substantially larger risk multipliers [Torres et al. 2016; James et al. 2006]. Cumulative exposure in premature infants [Fewtrell et al. 2009]—who receive both parenteral aluminum from nutrition solutions and vaccine-derived aluminum—has been associated with worse neurodevelopmental outcomes compared with lower-exposure peers, further supporting the gradient criterion.

Thus, the Bradford Hill criterion of *biological gradient,* or *dose-response* relationship, is demonstrated as well.

6. *Plausibility*: Aluminum is known to be phagocytosed by antigen-presenting cells and to activate the NLRP3 inflammasome [Eisenbarth et al. 2008; Li et al. 2008; Kool et al. 2008], which in turn drives caspase-1 activation and IL-1β maturation. IL-1β can alter endothelial tight junctions [Huppert et al. 2010; Argaw et al. 2012] upregulate adhesion molecules, and recruit additional immune cells, compromising Blood-Brain Barrier integrity and enabling

peripheral immune components to enter the brain. Complement proteins, particularly C1q and C3, label synapses for elimination [Stevens et al. 2007], and microglia remove these tagged synapses [Schafer et al. 2012] in an activity-dependent manner; excessive complement activation during critical developmental windows leads to aberrant pruning and circuit dysfunction [Stephan et al. 2012]. CD8+ T-cells, once inside the CNS, can release granzyme B and perforin [Henkart et al. 1985] into astrocytes and neurons, injuring astrocytic endfeet along vessel walls and disrupting glutamate homeostasis at the CSF–brain interface [DiStasio et al. 2019; Vargas et al. 2005].

This mechanistic chain is entirely compatible with known immunology and neurobiology, and each step is supported by peer-reviewed experimental work, demonstrating the Bradford Hill criterion of *plausibility* applies here.

7. *Coherence:* Aluminum's role as a potent activator of innate immunity via NLRP3 is widely accepted and has been replicated in multiple species [Eisenbarth et al. 2008; Li et al. 2008]. Brain inflammation, microglial activation, and lymphocytic infiltration are increasingly recognized features of ASD neuropathology, rather than speculative additions [Vargas et al. 2005; DiStasio et al. 2019]. HLA and other immune-related genetic variants (MTHFR and GST, etc.) have long been implicated in both autoimmunity and neurodevelopmental conditions, providing a plausible bridge between immune triggers and ASD risk [Torres et al. 2016; James et al. 2006; Buyske et al. 2006].

Clinical observations of immune dysregulation, gastrointestinal inflammation, and antibody abnormalities in ASD fit naturally into this immunogenetic framework rather than opposing it [Ashwood et al. 2011; Vargas et al. 2005; Frye 2015]. The Bradford Hill criterion of *coherence* is clearly met.

8. *Experimental Evidence:* Maternal immune activation models using cytokines, poly(I:C), or combinations of inflammatory and environmental triggers produce offspring with ASD-like behaviors, cortical maldevelopment, and persistent immune abnormalities, while manipulation of IL-6 or IL-17A signaling can attenuate or prevent these phenotypes [Choi et al. 2016; Smith et al. 2007].

Models showing HLA-like or immune genes show exaggerated responses to inflammatory challenges, providing direct evidence for gene–environment interactions [Hsiao and Patterson 2012; Hsiao et al. 2012]. Reversal or mitigation studies using anti-IL-1β agents, and other targeted interventions in animals demonstrate that intervening in the proposed pathway can reduce or normalize neuropathology, a strong experimental support for causation [Girard et al. 2010].

These multiple experimental models provide copious material demonstrating that the Bradford Hill criterion of *experimental evidence* is met in this instance.

9. *Analogy*: Toxic metals such as lead and mercury cause neurodevelopmental impairment through neuroinflammation, oxidative stress, and disrupted synaptic pruning, all of which

overlap mechanistically with the aluminum–NLRP3–complement pathway [Grandjean & Landrigan 2014; Modabbernia et al. 2017]. Maternal infections and systemic inflammatory states during pregnancy are associated with increased ASD risk and are mechanistically linked to cytokine-driven alterations of fetal brain development [Atladottir et al. 2012; Choi et al. 2016].

Furthermore, the literature on Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA) describes adjuvant-triggered autoimmune and neurological syndromes in genetically susceptible individuals, establishing that adjuvants can drive clinically significant immunopathology [Shoenfeld et al. 2011; Watad et al. 2018]. Other inflammogens, such as lipopolysaccharide and poly(I:C), produce similar patterns of microglial activation, synaptic loss, and behavioral change in developmental models, reinforcing the analogy between diverse pro-inflammatory exposures and ASD-like outcomes [Smith et al. 2007; Hsiao et al. 2012].

Clearly, with other heavy metals serving as analogs to aluminum exposure, maternal inflammatory states during pregnancy serving as analogs to ASD causation, and ASIA serving as an analog adjuvant-induced clinical syndrome, the Bradford Hill criterion of *analogy* is definitively met.

In sum, when reviewed in detail, this proposed mechanism of aluminum adjuvants in the pathogenesis of ASD meets *all nine* of the Bradford Hill criteria for causation, which is strongly indicative of a causal relationship.

CHAPTER 7: CONCLUSION

The autism spectrum disorder (ASD) crisis confronting global public health in 2025—with prevalence at 1 in 31 children [Shaw et al., 2025] and cumulative lifetime costs exceeding $460 billion annually in the United States alone [Leigh & Du, 2015]—demands a response grounded in scientific rigor, epidemiological honesty, and regulatory accountability. The preceding six chapters establish a comprehensive mechanistic, neuropathological, genetic, and legal framework linking aluminum adjuvant exposure to ASD in genetically susceptible individuals through NLRP3 inflammasome activation, IL-1β-mediated blood-brain barrier disruption, T-lymphocyte infiltration, astrocyte dysfunction, and complement-mediated dysregulation of synaptic pruning. This evidence satisfies all nine Bradford Hill criteria to support causation, and aligns with established principles of toxicology, immunology, and neurodevelopment [Tomljenovic & Shaw 2011; Grandjean & Landrigan 2014; Vallese et al. 2024; Wei et al. 2011; Saghazadeh et al. 2019].

Yet this robust body of mechanistic and neuropathological evidence remains systematically marginalized within mainstream vaccine safety discourse—not because it lacks scientific merit, but because it confronts structural barriers embedded in the institutions charged with safeguarding public health. These barriers include regulatory capture by industry interests [Carpenter & Moss, 2014; Abraham, 2002; Maynard & Bloor, 2015], reliance on population-level epidemiology incapable of detecting subgroup vulnerabilities [DeLong, 2011; Institute of Medicine, 2012], peer review gatekeeping that privileges null findings

over mechanistic discovery [Ioannidis, 2022; Horton, 2015], and legal frameworks that shield manufacturers from accountability while denying compensation to injured children [IOM, 2010; Bruesewitz v. Wyeth, 2011].

Other vaccine components and delivery factors may also contribute to neurodevelopmental risk in susceptible populations and warrant inclusion in causation discussions. These include: (1) mercury from thimerosal (ethylmercury), historically present in multi-dose vials and until very recently still used in some influenza vaccines, which has been linked to mitochondrial dysfunction, oxidative stress, and neuroinflammation in animal and cell models [Geier et al., 2009; Kern et al., 2016]; (2) live attenuated viruses (e.g., in MMR), which can induce transient immune activation and molecular mimicry in genetically predisposed children [Singh et al., 2002]; (3) other adjuvants such as squalene (in some formulations) and AS03/AS04 oil-in-water emulsions, which may amplify systemic inflammation [Shaw et al., 2013]; (4) combination effects of multiple simultaneous antigens and adjuvants at well-child visits, potentially overwhelming immature immune and detoxification systems [DeLong, 2011]; and (5) residual contaminants or manufacturing byproducts (e.g., polysorbate 80, formaldehyde traces), which have been hypothesized to disrupt blood-brain barrier integrity or glial function in early development [Shaw & Tomljenovic, 2013]. These additional factors do not diminish the central role of aluminum but suggest that the total immunologic and toxicologic burden of the schedule—rather than any single component—may be the critical driver in genetically susceptible children.

The convergence of mechanistic, neuropathological, genetic, and epidemiological lines of evidence around aluminum exposure however, paints a compelling yet disturbing picture where the exquisitely timed processes of synaptic pruning, myelination, and circuit refinement are disrupted during the critical 3-month to 3-year developmental window. The resulting neuropathology provides a coherent biological substrate for the core ASD phenotype: impaired social communication networks, sensory hypersensitivity, repetitive behaviors, and motor/coordination deficits. While no single study proves causation in isolation, the satisfaction of all nine Bradford Hill criteria across independent lines of inquiry, together with reproducible animal-model reversibility and human postmortem findings, demands urgent, transparent, and rigorous re-examination of aluminum adjuvant safety in the context of early childhood neurodevelopment. The scale of the current ASD crisis—now affecting 1 in 31 children with societal costs approaching half a trillion dollars annually—requires nothing less than a complete paradigm shift in how we evaluate and regulate vaccine components that enter the developing nervous system during its most vulnerable period.

REFERENCES

Abdolmaleky HM, Martin M, Zho JR., Thiagalingam, S. (2023). Epigenetic Alterations of Brain Non-Neuronal Cells in Major Mental Diseases. *Genes*, *14*(4), 896. Doi:10.3390/genes14040896.

Abraham, J. (2002). The pharmaceutical industry as a political player. *Lancet*. Doi:10.1016/S0140-6736(02)11477-2.

Alyautdin RN, Petrov VE, Langer K, Berthold A, Kharkevich DA, Kreuter J. (1997). Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles. *Pharmaceutical Research 14*(3), 325-8. Doi:10.1023/a:1012098005098.

Ameis SH, Lerch JP, Taylor MJ, Lee W, et al. (2016). A diffusion tensor imaging studyin children with ADHD, autism spectrum disorder, OCD, and matched controls: Distinct and non-distinct white matter disruption and dimensional brain-behavior relationships. *American Journal of Psychiatry 173*(12), 1213-1222. Doi:10.1176/appi.ajp.2016.15111435.

American Psychiatric Association. (1980). Diagnostic and statistical manual of mental disorders (3rd ed.). https://www.terapiacognitiva.eu/dwl/dsm5/DSM-III.pdf.

American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders (4th ed.). https://img3.reoveme.com/m/2ab8dabd068b16a5.pdf.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). https://www.medialook.al/wp-content/uploads/2020/03/DSM-5-By-American-Psychiatric-Association.pdf

Anashkina AA, Erlykina E I. (2021). Molecular Mechanisms of Aberrant Neuroplasticity in Autism Spectrum Disorders (Review). *Sovremennye tekhnologii v Meditsine 13*(1), 78–91. Doi: 10.17691/stm2021.13.1.10.

Angrand L, Gherardi RK, Crépeaux G. (2025). Regulatory limits of aluminium content of vaccines have not been set based on toxicological studies. *Environmental Toxicology and Pharmacology 119*, 104812. Doi:10.1016/j.etap.2025.104812.

Angrand L, Masson JD, Rubio-Casillas A, Nosten-Bertrand M, Crépeaux G. (2022). Inflammation and Autophagy: A Convergent Point between Autism Spectrum Disorder (ASD)-Related Genetic and Environmental Factors: Focus on Aluminum Adjuvants. *Toxics 10*(9), 518. Doi:10.3390/toxics10090518.

Araszkiewicz AF, Jańczak K, Wójcik P, Białecki B, et al. (2025). MTHFR Gene Polymorphisms: A Single Gene with Wide-Ranging Clinical Implications—A Review. *Genes16*(4), 441–1. Doi:10.3390/genes16040441.

Argaw AT, Asp L, Zhang J, Navrazhina K, et al. (2012). Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *The Journal of Clinical Investigation 122*(7), 2454–2468. Doi:10.1172/JCI60842.

Argaw AT, Zhang Y, Snyder BJ, Zhao ML, Kopp N, Lee SC, Raine CS, Brosnan CF, John GR. (2006). IL-1beta regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. *Journal of Immunology (Baltimore, Md.:1950) 177*(8), 5574–5584. Doi:10.4049/jimmunol.177.8.5574.

Arumugham, V. (2017). Safety studies of aluminum in vaccines lack immunotoxicity analysis of this immunological adjuvant: Ignorance or deception? *Zenodo*. Doi:10.5281/zenodo.1117242.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, et al. (2011). Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, Behavior, and Immunity 25*(1), 40–45. Doi:10.1016/j.bbi.2010.08.003.

Atladóttir HÓ, Henriksen TB, Schendel DE, Parner ET. (2012). Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics, 130*(6), e1447–e1454. Doi:10.1542/peds.2012-1107.

Bai D, Yip BHK, Windham, GC, Sourander A, et al. (2019). Association of Genetic and Environmental Factors With Autism in a 5-Country Cohort. *JAMA Psychiatry 76*(10), 1035–1043. Doi:10.1001/jamapsychiatry.2019.1411.

Bairwa SC, Shaw CA, Kuo M, Yoo J, Tomljenovic L, Eidi H. (2021). Cytokines profile in neonatal and adult wild-type mice post-injection of the U.S. pediatric vaccination schedule. *Brain, Behavior, & Immunity - Health 15*, 100267. Doi:10.1016/j.bbih.2021.100267.

Baldwin GS, Carnegie PR. (1971). Specific enzymic methylation of an arginine in the experimental allergic encephalomyelitis protein from human myelin. *Science (New York, N.Y.), 171*(3971), 579–581. Doi:10.1126/science.171.3971.579.

Barger BD, Campbell JM, McDonough JD. (2013). Prevalence and onset of regression within autism spectrum disorders: a meta-analytic review. *Journal of Autism and Developmental Disorders 43*(4), 817–828. Doi:10.1007/s10803-012-1621-x.

Bayer SA, Altman J, Russo RJ, Zhang X. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology 14*(1), 83–144. https://neurondevelopment.org/wp-content/uploads/2023/10/Bayer-et-al-Neurotox-14-1993.pdf.

Baylor NW, Egan W, Richman P. (2002). Aluminum salts in vaccines--US perspective. *Vaccine, 20 Suppl 3*, S18–S23. Doi:10.1016/s0264-410x(02)00166-4.

Besag F. (2017). Epilepsy in patients with autism: Links, risks and treatment challenges. *Neuropsychiatric Disease and Treatment, 14*(14),1–10. Doi:10.2147/NDT.S120509.

Blank M, Barzilai O, Shoenfeld Y. (2007). Molecular mimicry and auto-immunity. *Clinical Reviews in Allergy & Immunology 32*(1), 111–118. Doi:10.1007/BF02686087.

Blaylock R. (2015). Additive aluminum as a cause of induced immunoexcitoxicity resulting in neurodevelopmental and neurodegenerative disorders: A biochemical, pathophysiological, and pharmacological analysis. *Surgical Neurology International*. Doi:10.25259/SNI_296_2024.

Blaylock RL. (2024). Additive aluminum as a cause of induced immunoexcitotoxicity resulting in neurodevelopmental and neurodegenerative disorders: A biochemical, pathophysiological, and pharmacological analysis. *Surgical Neurology International 15*, 171. Doi:10.25259/SNI_296_2024.

Boat TF, Wu JT, editors. (2015). Prevalence of autism spectrum disorder. In Committee to Evaluate the Supplemental Security Income Disability Program for Children with Mental Disorders; Board on the Health of Select Populations; Board on Children, Youth, and Families; Institute of Medicine; National Academies of Sciences, Engineering, and Medicine (Eds.), Mental disorders and disabilities among low-income children (Chapter 14). National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK332896/.

Bolton SJ, Anthony DC, Perry VH. (1998). Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. *Neuroscience 86*(4), 1245–1257. Doi:10.1016/s0306-4522(98)00058-x.

Boretti A. (2021). Reviewing the association between aluminum adjuvants in the vaccines and autism spectrum disorder. *Journal of Trace Elements in Medicine and Biology: Organ of the Society for Minerals and Trace Elements (GMS) 66*, 126764. Doi:10.1016/j.jtemb.2021.126764.

Boris M, Goldblatt A, Galanko J, James SJ. (2004). "Association of MTHFR gene variants with autism." *Journal of American Physicians and Surgeons 9*(4),106. www.jpands.org/vol9no4/boris.pdf.

Bourgeois JP, Rakic P. (1993). Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 13*(7), 2801–2820. Doi:10.1523/JNEUROSCI.13-07-02801.1993.

Braunschweig D, Krakowiak P, Duncanson P, Boyce R, et al. (2013). Autism-specific maternal autoantibodies recognize critical proteins in developing brain. *Translational Psychiatry 3*(7), e277. Doi:10.1038/tp.2013.50.

Brimberg L, Mader S, Jeganathan V, Berlin R, et al. (2016). Caspr2-reactive antibody cloned from a mother of an ASD child mediates an ASD-like phenotype in mice. *Molecular Psychiatry 21*(12), 1663–1671. Doi:10.1038/mp.2016.165.

Bruesewitz v. Wyeth LLC, 562 U.S. 223 (2011). https://supreme.justia.com/cases/federal/us/562/223/.

Buckland, R. (2024). The Buckland Review of Autism Employment: Report and recommendations. Department for Work and Pensions.

https://www.gov.uk/government/publications/the-buckland-review-of-autism-employment-report-and-recommendations/the-buckland-review-of-autism-employment-report-and-recommendations.
Buescher AVS, Cidav Z, Knapp M, Mandell DS. (2014). Costs of Autism Spectrum Disorders in the United Kingdom and the United States. *JAMA Pediatrics*, *168*(8), 721. Doi:10.1001/jamapediatrics.2014.210.

Buyske S, Williams TA, Mars AE, Stenroos ES, et al. (2006). Analysis of case-parent trios at a locus with a deletion allele: association of GSTM1 with autism. *BMC Genetics 7*, 8. Doi:10.1186/1471-2156-7-8.

Cakir J, Frye RE, Walker SJ. (2020). The lifetime social cost of autism: 1990–2029. *Research in Autism Spectrum Disorders (72)*, 101502. Doi:10.1016/j.rasd.2019.101502.

Carpenter D, Moss DA. (2014). Introduction. In D. Carpenter & D. A. Moss (Eds.), Preventing regulatory capture: Special interest influence and how to limit it (pp. 1–22). Cambridge University Press. Doi:10.1017/CBO9781139565875.002.

Centers for Disease Control and Prevention. (2025). Data and statistics on autism spectrum disorder. https://www.cdc.gov/autism/data-research/index.html.

Choi GB, Yim YS, Wong H, et al. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science 351*(6276), 933-939. Doi:10.1126/science.aad0314.

Chung WS, Allen NJ, Eroglu C. (2015). Astrocytes Control Synapse Formation, Function, and Elimination. *Cold Spring Harbor Perspectives in Biology 7*(9). Doi:10.1101/cshperspect.a020370.

Clark IA, Vissel B. (2016). Excess cerebral TNF causing glutamate excitotoxicity rationalizes treatment of neurodegenerative diseases and neurogenic pain by anti-TNF agents. *Journal of Neuroinflammation 13*, 236. Doi:10.1186/s12974-016-0708-2.

Constituent materials, 21 C.F.R. § 610.15 (2025). https://www.ecfr.gov/current/title-21/part-610/section-610.15

Crépeaux G, Eidi H, David MO, Baba-Amer Y, Tzavara E, Giros B, Authier FJ, Exley C, Shaw CA, Cadusseau J, Gherardi RK. (2017). Non-linear dose-response of aluminium hydroxide adjuvant particles: Selective low dose neurotoxicity. *Toxicology*, *375*, 48–57. Doi:10.1016/j.tox.2016.11.018.

Crépeaux G, Gherardi RK, Authier FJ. (2018). ASIA, chronic fatigue syndrome, and selective low dose neurotoxicity of aluminum adjuvants. *The Journal of Allergy and Clinical Immunology. in Practice 6*(2), 707. Doi:10.1016/j.jaip.2017.10.039.

DeLong, G. (2011). A Positive Association found between Autism Prevalence and Childhood Vaccination uptake across the U.S. Population. *Journal of Toxicology and Environmental Health, Part A*, *74*(14), 903–916. Doi:10.1080/15287394.2011.573736.

Deoni SC, Dean DC 3rd, O'Muircheartaigh J, Dirks H, et al. (2012). Investigating white matter development in infancy and early childhood using myelin water faction and relaxation time mapping. *NeuroImage 63*(3), 1038–1053. Doi:10.1016/j.neuroimage.2012.07.037.

Deoni SC, Mercure E, Blasi A , Gasston D, et al. (2011). Mapping infant brain myelination with magnetic resonance imaging. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 31*(2), 784–791. Doi:10.1523/JNEUROSCI.2106-10.2011.

Deoni SC, O'Muircheartaigh J, Elison JT, Walker L, et al. (2016). White matter maturation profiles through early childhood predict general cognitive ability. *Brain Structure & Function, 221*(2), 1189–1203. Doi:10.1007/s00429-014-0947-x.

DiStasio MM, Nagakura I, Nadler MJ, Anderson MP. (2019). T lymphocytes and cytotoxic astrocyte blebs correlate across autism brains. *Annals of Neurology 86*(6), 885–98. Doi:10.1002/ana.25610.

Edmonson C, Ziats MN, Rennert OM. (2014). Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. *Molecular Autism 5*(1), 3. Doi:10.1186/2040-2392-5-3.

Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, et al. (2008). Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature 453*(7198), 1122–1126. Doi:10.1038/nature06939.

El-Ansary A, Al-Ayadhi L. (2014). GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders. *Journal of Neuroinflammation 11*, 189. Doi:10.1186/s12974-014-0189-0.

Ellul P, Rosenzwajg M, Peyre H, Fourcade G, et al. (2021). Regulatory T lymphocytes/Th17 lymphocytes imbalance in autism spectrum disorders: evidence from a meta-analysis. *Molecular Autism 12*(1), 68. Doi:10.1186/s13229-021-00472-4.

Enstrom AM, Onore CE, Van de Water JA, Ashwood P. (2010). Differential monocyte responses to TLR ligands in children with autism spectrum disorders. *Brain, Behavior, and Immunity 24*(1), 64–71. Doi:10.1016/j.bbi.2009.08.001.

European Chemicals Agency. (2015). Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance (Version 4.1). https://echa.europa.eu/documents/10162/17224/information_requirements_r7a_en.pdf.

Fewtrell MS, Bishop NJ, Edmonds CJ, Isaacs EB, et al. (2009). Aluminum exposure from parenteral nutrition in preterm infants: bone health at 15-year follow-up. *Pediatrics 124*(5), 1372–1379. Doi:10.1542/peds.2009-0783.

Fischer I, Shohat S, Leichtmann-Bardoogo Y, Nayak R, et al. (2024). Shank3 mutation impairs glutamate signaling and myelination in ASD mouse model and human iPSC-derived OPCs. *Science Advances 10*(41), eadl4573. Doi:10.1126/sciadv.adl4573.

Flarend RE, Hem SL, White JL, Elmore D, Suckow MA, Rudy AC, Dandashli EA. (1997). In vivo absorption of aluminium-containing vaccine adjuvants using 26Al. *Vaccine 15*(12-13), 1314-1318. Doi:10.1016/s0264-410x(97)00041-8.

Franchi L, Eigenbrod T, Muñoz-Planillo R, Nuñez G. (2009). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nature Immunology 10*(3), 241–7. Doi:10.1038/ni.1703.

Franchi L, Núñez G. (2008). The Nlrp3 inflammasome is critical for aluminium hydroxide-mediated IL-1β secretion but dispensable for adjuvant activity. *European Journal of Immunology 38*(8), 2085–9. Doi:10.1002/eji.200838549.

Frye RE, Rose S, Slattery J, MacFabe DF. (2015). Gastrointestinal dysfunction in autism spectrum disorder: the role of the mitochondria and the enteric microbiome. *Microbial Ecology in Health and Disease 26*, 27458. Doi:10.3402/mehd.v26.27458.

Frye RE, Sequeira JM, Quadros EV, James SJ, et al. (2013). Cerebral folate receptor autoantibodies in autism spectrum disorder. *Molecular Psychiatry 18*(3), 369–38. Doi:10.1038/mp.2011.175.

Ganz ML. (2007). The Lifetime Distribution of the Incremental Societal Costs of Autism. *Archives of Pediatrics & Adolescent Medicine, 161*(4), 343. Doi:10.1001/archpedi.161.4.343.

Gao M, Mei D, Huo Y, Mao S. (2019). Effect of polysorbate 80 on the intranasal absorption and brain distribution of tetramethylpyrazine phosphate in rats. *Drug Delivery and Translational Research, 9*(1), 311–318. Doi:10.1007/s13346-018-0580-y.

Garner J. (2022). The Control Group Pilot Study of Unvaccinated vs. Vaccinated Americans. *International Journal of Vaccine Theory, Practice, and Research 3*(1), 862-913. Doi:10.56098/ijvtpr.v3i1.45.

Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Geier MR. (2009). A prospective study of transsulfuration biomarkers in autistic disorders. *Neurochemical Research 34*(2), 386–393. Doi:10.1007/s11064-008-9782-x.

Gherardi RK, Eidi H, Crépeaux G, Authier FJ, Cadusseau J. (2015). Biopersistence and Brain Translocation of Aluminum Adjuvants of Vaccines. *Frontiers in Neurology, 6*, 4. Doi:10.3389/fneur.2015.00004.

Girard S, Tremblay L, Lepage M, Sébire G. (2010). IL-1 receptor antagonist protects against placental and neurodevelopmental defects induced by maternal inflammation. *Journal of Immunology (Baltimore, Md.:1950) 184*(7), 3997–4005. Doi:10.4049/jimmunol.0903349.

Goldman, GS, Cheng RZ. (2025). The Immature Infant Liver: Cytochrome P450 Enzymes and their Relevance to Vaccine Safety and SIDS Research. *International Journal of Medical Sciences*, 22(10), 2434-2445. Doi:10.7150/ijms.114402.

Grandjean P, Landrigan PJ. (2014). Neurobehavioural effects of developmental toxicity. *Lancet Neurology 13*(3), 330-8. Doi:10.1016/S1474-4422(13)70278-3.

Grotheer M, Rosenke M, Wu H, Kular H, et al. (2022). White matter myelination during early infancy is linked to spatial gradients and myelin content at birth. *Nature Communications 13*(1), 997. Doi:10.1038/s41467-022-28326-4.

Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J. (1999). Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. *Pharmaceutical Research 16*(10), 1564-9. Doi:10.1023/a:1018983904537.

Gunaydin M, Dogan O, Gunay F, Cikili-Uytun M, Celik-Buyukceran Ö, Oztop DB. (2025). Complement system dysfunction in autism spectrum disorder: evidence for altered C1q and C3 levels (complement system dysfunction in ASD). *Acta Neuropsychiatrica 37,* e64. Doi:10.1017/neu.2025.10017.

Haroon E, Miller A, Sanacora, G. (2017). Inflammation, Glutamate, and Glia: A Trio of Trouble in Mood Disorders. *Neuropsychopharmacology 42*, 193–215. Doi:10.1038/npp.2016.199.

Harte C, Gorman AL, McCluskey S, Carty M, et al. (2017). Alum Activates the Bovine NLRP3 Inflammasome. *Frontiers in Immunology 8*, 1494. Doi:10.3389/fimmu.2017.01494.

Hassan AH, Salem AM, Shehab AA, El Hossiny RM. (2025). Diagnostic and severity correlation of serum zinc, copper, and anti-myelin basic protein antibodies in children with autism spectrum disorder: a cross-sectional controlled study. *Egyptian Pediatric Association Gazette, 73.* Doi:10.1186/s43054-025-00406-2.

Henkart PA. (1985). Mechanism of lymphocyte-mediated cytotoxicity. *Annual Review of Immunology 3*, 31–58. Doi:10.1146/annurev.iy.03.040185.000335.

Hill AB. (1965). The Environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, *58*(5), 295–300. Doi:10.1177/003591576505800503.

HogenEsch, H. (2013). Mechanism of Immunopotentiation and Safety of Aluminum Adjuvants. *Frontiers in Immunology, 3*(406). Doi:10.3389/fimmu.2012.00406.

Hooker BS, Miller NZ. (2020). Analysis of health outcomes in vaccinated and unvaccinated children: Developmental delays, asthma, ear infections and gastrointestinal disorders. *SAGE Open Medicine 8*. Doi:10.1177/2050312120925344.

Hooker BS, Miller NZ. (2021). Health effects in vaccinated versus unvaccinated children, with covariates for breastfeeding status and type of birth. *Journal of Translational Science 7*(1),1-11. Doi:10.15761/JTS.1000459.

Hornung V, Bauernfeind F, Halle A, Samstag E, Kono H, Rock K, Fitzgerald K, Latz E. (2008). "Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization." *Nature Immunology 9*(8), 847–856. Doi:10.1038/ni.1631.

Horton, R. (2015). Offline: What is medicine's 5 sigma? *The Lancet 385*(9976), 1380. Doi:10.1016/S0140-6736(15)60696-1.

Hsiao EY, McBride SW, Chow J, Mazmanian SK, et al. (2012). Modeling an autism risk factor in mice leads to permanent immune dysregulation. *Proceedings of the National Academy of Sciences of the United States of America 109*(31), 12776–12781. Doi:10.1073/pnas.1202556109.

Hsiao EY, Patterson PH. (2012). Placental regulation of maternal-fetal interactions and brain development. *Developmental Neurobiology 72*(10), 1317–1326. Doi:10.1002/dneu.22045.

Huppert J, Closhen D, Croxford A, White R, et al. (2010). Cellular mechanisms of IL-17-induced blood-brain barrier disruption. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 24*(4), 1023–1034. Doi:10.1096/fj.09-141978.

Hutsler JJ, Zhang H. (2010). Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Research 1309*, 83–94. Doi:10.1016/j.brainres.2009.09.120.

Huttenlocher PR, Dabholkar AS. (1997). Regional differences in synaptogenesis in human cerebral cortex. *The Journal of Comparative Neurology 387*(2), 167–178. Doi: 10.1002/(sici)1096-9861(19971020)387:2<167::aid-cne1>3.0.co;2-z.

Huynh W, Cordato DJ, Kehdi E, Masters LT, Dedousis C. (2008). Post-vaccination encephalomyelitis: literature review and illustrative case. *Journal of Clinical Neuroscience 15*(12), 1315–1322. Doi:10.1016/j.jocn.2008.05.002.

Institute of Medicine (US) Committee on Review of Adverse Effects of Vaccines. (2012). Adverse Effects of Vaccines: Evidence and Causality. National Academies Press (US). https://www.nationalacademies.org/projects/PHPH-H-08-17-A.

Institute of Medicine (US) Committee to Review Adverse Effects of Vaccines. (2012). Adverse Effects of Vaccines: Evidence and Causality. Washington (DC): National Academies Press. Summary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK190010/ncbi.nlm.nih+1.

Institute of Medicine (US) Committee on Review of Priorities in the National Vaccine Plan. (2010). 1986 National Childhood Vaccine Injury Act (Public Law 99-660). In Priorities for the National Vaccine Plan. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK220067/.

Ioannidis JPA. (2016). Why Most Clinical Research Is Not Useful. *PLOS Medicine 13*(6), e1002049. Doi:10.1371/journal.pmed.1002049.

Jaldin-Fincati JR, Moussaoui S, Gimenez MC, Ho CY, Lancaster CE, Botelho RJ, Lancaster CE, Botelho RJ, Ausar SF, Brookes RH, Terebiznik MR. (2022). Aluminum hydroxide adjuvant diverts the uptake and trafficking of genetically detoxified pertussis toxin to lysosomes in macrophages. *Molecular Microbiology 117*(5), 1173–95. Doi:10.1111/mmi.14900.

James SJ, Cutler P, Melnyk S, Jernigan S, et al. (2004). Metabolic Biomarkers of Increased Oxidative Stress and Impaired Methylation Capacity in Children with Autism. *The American Journal of Clinical Nutrition 80(6)*, 1611–7. Doi:10.1093/ajcn/80.6.1611.

James SJ, Melnyk S, Jernigan S, Cleves MA, et al. (2006). Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics 141B*(8), 947–956. Doi:10.1002/ajmg.b.30366.

Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. The complement system and innate immunity. https://www.ncbi.nlm.nih.gov/books/NBK27100/.

Johnson WG, Buyske S, Mars AE, Sreenath M, Set al. (2009). HLA-DR4 as a risk allele for autism acting in mothers of probands possibly during pregnancy. *Archives of Pediatrics & Adolescent Medicine 163*(6), 542–546. Doi:10.1001/archpediatrics.2009.74.

Jones JP III, Williamson L, Konsoula Z, Anderson R, et al. (2024). Evaluating the Role of Susceptibility Inducing Cofactors and of Acetaminophen in the Etiology of Autism Spectrum Disorder. *Life 14*(8), 918. Doi:10.3390/life14080918.

Just MA, Keller TA, Malave VL, Kana RK, et al. (2012). Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. *Neuroscience and Biobehavioral Reviews 36*(4), 1292–1313. Doi:10.1016/j.neubiorev.2012.02.007.

Kaur A, Jairath M, Kaur A. (2023). Heavy metals and genetic variations in folate metabolism pathway: A gene-environment interaction. *Indian Journal of Public Health 67*(3), 477–479. Doi:10.4103/ijph.ijph_445_22.

Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, et al. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nature Medicine 13*(10), 1173–1175. Doi:10.1038/nm1651.

Keown CL, Shih P, Nair A, Peterson N, et al. (2013). Local functional overconnectivity in posterior brain regions is associated with symptom severity in autism spectrum disorders. *Cell Reports 5*(3), 567–572. Doi:10.1016/j.celrep.2013.10.003.

Kern JK, Geier DA, Sykes LK, & Geier MR. (2016). Relevance of Neuroinflammation and Encephalitis in Autism. *Frontiers in Cellular Neuroscience 9*, 519. Doi:10.3389/fncel.2015.00519.

Khan Z, Combadière C, Authier FJ, Itier V, Lux F, Exley C, Mahrouf-Yorgov M, Decrouy X, Moretto P, Tillement O, Gherardi RK, Cadusseau J. (2013). Slow CCL2-dependent translocation of biopersistent particles from muscle to brain. *BMC Medicine*, *11*, 99. Doi:10.1186/1741-7015-11-99.

Kim SR, Kim DI, Kim SH, Lee H, Lee KS, Cho SH, et al. (2014). NLRP3 inflammasome activation by mitochondrial ROS in bronchial epithelial cells is required for allergic inflammation. *Cell Death & Disease 5*(10), e1498–8. Doi:10.1038/cddis.2014.460.

King M, Bearman P. (2009). Diagnostic change and the increased prevalence of autism. *International Journal of Epidemiology 38*(5), 1224–1234. Doi:10.1093/ije/dyp261.

Kivity S, Agmon-Levin N, Blank M, Shoenfeld Y. (2009). Infections and autoimmunity – friends or foes? *Trends in Immunology 30*(8), 409–414. Doi:10.1016/j.it.2009.05.005.

Kool M, Pétrilli V, De Smedt T, Rolaz A, et al. (2008). Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *Journal of Immunology (Baltimore, Md.: 1950) 181*(6), 3755–3759. Doi:10.4049/jimmunol.181.6.3755.

Korn T, Bettelli E, Oukka M, Kuchroo VK. (2009). IL-17 and Th17 Cells. *Annual Review of Immunology 27*, 485–517. Doi:10.1146/annurev.immunol.021908.132710.

Korn T, Oukka M, Kuchroo V, Bettelli E. (2007). Th17 cells: effector T cells with inflammatory properties. *Seminars in Immunology 19*(6), 362–371. Doi:10.1016/j.smim.2007.10.007.

Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina, SE, Engelhardt B, Alyautdin R, von Briesen, H, Begley DJ. (2003). Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. *Pharmaceutical Research 20*(3), 409–416. Doi:10.1023/a:1022604120952.

Lau A, Tymianski M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Arch - Eur J Physiol 460*, 525–542. Doi:10.1007/s00424-010-0809-1.

Lavelle TA, Weinstein MC, Newhouse JP, Munir K, Kuhlthau KA, Prosser LA. (2014). Economic Burden of Childhood Autism Spectrum Disorders. *Pediatrics*, *133*(3), e520–9. Doi:10.1542/peds.2013-0763.

Leigh JP, Du J. (2015). Brief Report: Forecasting the Economic Burden of Autism in 2015 and 2025 in the United States. *Journal of Autism and Developmental Disorders*, *45*(12), 4135–9. Doi:10.1007/s10803-015-2521-7.

Li B, Zhang X, Huo S, Zhang J, Du J, Xiao B, Song M, Shao B, Li Y. (2022). Aluminum activates NLRP3 inflammasome-mediated pyroptosis via reactive oxygen species to induce liver injury in mice. *Chemico-Biological Interactions 368*, 110229. Doi:10.1016/j.cbi.2022.110229.

Li H, Willingham SB, Ting JPY, Re F. (2008). Cutting Edge: Inflammasome Activation by Alum and Alum's Adjuvant Effect Are Mediated by NLRP3. *The Journal of Immunology181*(1), 17–21. Doi:10.4049/jimmunol.181.1.17.

Li J, Zhang L, Chu Y, Namaka M, et al. (2016). Astrocytes in Oligodendrocyte Lineage Development and White Matter Pathology. Frontiers in Cellular Neuroscience 10, 119. Doi:10.3389/fncel.2016.00119.

Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M. (2009). Elevated immune response in the brain of autistic patients. *Journal of Neuroimmunology 207*(1-2), 111–116. Doi:10.1016/j.jneuroim.2008.12.002.

Liao X, Liu Y, Fu X, Li Y. (2020). Postmortem Studies of Neuroinflammation in Autism Spectrum Disorder: a Systematic Review. *Molecular Neurobiology 57*(8), 3424–3438. Doi:10.1007/s12035-020-01976-5.

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature 541*(7638), 481–487. Doi:10.1038/nature21029.

Lim ET, Uddin M, De Rubeis S, Chan Y, et al. (2017). Rates, distribution and implications of postzygotic mosaic mutations in autism spectrum disorder. *Nature Neuroscience 20*(9), 1217–1224. Doi:10.1038/nn.4598.

Liu X, Ying J, Wang X, Zheng Q, Zhao T, Yoon S, et al. (2021). Astrocytes in Neural Circuits: Key Factors in Synaptic Regulation and Potential Targets for Neurodevelopmental Disorders. *Frontiers in Molecular Neuroscience 14*. Doi:10.3389/fnmol.2021.729273.

Lyons-Weiler J and Blaylock RL. (2022). Revisiting Excess Diagnoses of Illnesses and Conditions in Children Whose Parents Provided Informed Permission to Vaccinate Them. *International Journal of Vaccine Theory Practice and Research 2*(2), 603–18.10.56098/ijvtpr.v2i2.59.

Mandolesi G, Musella A, Gentile A, Grasselli G, Haji N, Sepman H, Fresegna D, Bullitta S, De Vito F, Musumeci G, Di Sanza C, Strata P, Centonze D. (2013). Interleukin-1β alters glutamate transmission at purkinje cell synapses in a mouse model of multiple sclerosis. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 33*(29), 12105–12121. Doi:10.1523/JNEUROSCI.5369-12.2013.

Mansur F, Silva T, Gomes AL, Magdalon AKS, et al. (2021). Complement C4 Is Reduced in iPSC-Derived Astrocytes of Autism Spectrum Disorder Subjects. *International Journal of Molecular Sciences 22*(14), 7579. Doi:10.3390/ijms22147579.

Marrack P, McKee AS, Munks MW. (2009). Towards an understanding of the adjuvant action of aluminium. *Nature Reviews Immunology*, *4*, 287–93. Doi:10.1038/nri2510.

Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. (2015). Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Molecular Psychiatry 20*(4), 440–446. Doi:10.1038/mp.2014.59.

Masson JD, Angrand L, Badran G, de Miguel R, Crépeaux G. (2022). Clearance, biodistribution, and neuromodulatory effects of aluminum-based adjuvants. Systematic review and meta-analysis: what do we learn from animal studies? *Critical Reviews in Toxicology 52*(6), 403–19. Doi:10.1080/10408444.2022.2105688.

Masson JD, Crépeaux G, Authier FJ, Exley C, Gherardi RK. (2017). Critical analysis of reference studies on the toxicokinetics of aluminum-based adjuvants. *Journal of Inorganic Biochemistry 181*, 87–95. Doi:10.1016/j.jinorgbio.2017.12.015.

Mawson AD, Ray BR, Bhuiyan A, Jacob B. (2017). Pilot comparative study on the health of vaccinated and unvaccinated 6- to 12- year old U.S. children. *Journal of Translational Science 3*(3). Doi:10.15761/JTS.1000186.

Mawson A, Jacob B. (2025). Vaccination and Neurodevelopmental Disorders: A Study of Nine-Year-Old Children Enrolled in Medicaid. *Science, Public Health Policy, and the Law 6*(1), 1-22. https://publichealthpolicyjournal.com/wp-content/uploads/2025/01/Mawson_Jacob_SciencePublicHealthPolicyAndTheLaw_v6.2019-2025.Jan_2025.pdf.

Maynard A, Bloor K. (2015). Regulation of the pharmaceutical industry: promoting health or protecting wealth?. *Journal of the Royal Society of Medicine 108*(6), 220–222. Doi:10.1177/0141076814568299.

McColl BW, Rothwell NJ, Allan SM. (2008). Systemic inflammation alters the kinetics of cerebrovascular tight junction disruption after experimental stroke in mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 28*(38), 9451–9462. Doi:10.1523/JNEUROSCI.2674-08.2008.

Mendiola AS, and Cardona AE. (2018). The IL-1β phenomena in neuroinflammatory diseases. *Journal of Neural Transmission (Vienna, Austria:1996) 125*(5), 781–795. Doi:10.1007/s00702-017-1732-9.

Meltzer A, Van de Water J. (2017). The Role of the Immune System in Autism Spectrum Disorder. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology 42*(1), 284–298. Doi:10.1038/npp.2016.158.

Miller NZ. (2016). Aluminum in Childhood Vaccines Is Unsafe. *Journal of American Physicians and Surgeons 21*(4), 109-117. https://www.researchgate.net/publication/311824598_Aluminum_in_Childhood_Vaccines_is_Unsafe.

Moaaz M, Youssry S, Elfatatry A, El Rahman MA. (2019). Th17/Treg cells imbalance and their related cytokines (IL-17, IL-10 and TGF-β) in children with autism spectrum disorder. *Journal of Neuroimmunology 337*, 577071. Doi:10.1016/j.jneuroim.2019.577071.

Modabbernia A, Velthorst E, Reichenberg A. (2017). Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. *Molecular Autism 8*, 13. Doi:10.1186/s13229-017-0121-4.

Montanari M, Martella G, Bonsi, Meringolo M. (2022). Autism Spectrum Disorder: Focus on Glutamatergic Neurotransmission. *International Journal of Molecular Sciences 23*(7), 3861. Doi:10.3390/ijms23073861.

Morgan JT, Chana G, Pardo CA, Achim C, et al. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry 68*(4), 368–376. Doi:10.1016/j.biopsych.2010.05.024.

Mostafa G, Shehab A. (2010). The link of C4B null allele to autism and to a family history of autoimmunity in Egyptian autistic children. *Journal of Neuroimmunology 223*(1-2), 115-119. Doi:10.1016/j.jneuroim.2010.03.025.

Muñoz-Planillo R, Kuffa P, Martínez-Colón G, Smith BL, Rajendiran TM, Núñez G. (2013). K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity 38*(6), 1142–1153. Doi:10.1016/j.immuni.2013.05.016.

Nevison CD. (2014). A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. *Environmental Health: A Global Access Science Source 13*, 73. Doi:10.1186/1476-069X-13-73.

Nevison CD, Blaxill M. (2017). Diagnostic Substitution for Intellectual Disability: A Flawed Explanation for the Rise in Autism. *Journal of Autism and Developmental Disorders 47*(9), 2733–2742. Doi:10.1007/s10803-017-3187-0.

Nicosia N, Giovenzana M, Misztak, Mingardi J, et al. (2024). Glutamate-Mediated Excitotoxicity in the Pathogenesis and Treatment of Neurodevelopmental and Adult Mental Disorders. *International Journal of Molecular Sciences 25*(12), 6521. Doi:10.3390/ijms25126521.

Odell D, Maciulis A, Cutler A, Warren L, et al. (2005). Confirmation of the association of the C4B null allelle in autism. *Human Immunology 66*(2), 140–145. Doi:10.1016/j.humimm.2004.11.002.

Ozonoff S, Iosif AM, Baguio F, Cook IC, et al. (2010). A prospective study of the emergence of early behavioral signs of autism. *Journal of the American Academy of Child and Adolescent Psychiatry 49*(3), 256–66.e662. https://pmc.ncbi.nlm.nih.gov/articles/PMC2923050/pdf/nihms224959.pdf.

Parker W, Anderson LG, Jones JP, Anderson R, et al. (2023). The Dangers of Acetaminophen for Neurodevelopment Outweigh Scant Evidence for Long-Term Benefits. *Children (Basel, Switzerland) 11*(1), 44. Doi:10.3390/children11010044.

Parker W, Hornik CD, Bilbo S, Holzknecht ZE, et al. (2017). The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. *The Journal of International Medical Research*, *45*(2) 407–438. Doi:10.1177/0300060517693423.

Pellerin L, Magistretti PJ. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proceedings of the National Academy of Sciences of the United States of America 91*(22), 10625–10629. Doi:10.1073/pnas.91.22.10625.

Perricone C, Colafrancesco S, Mazor RD, Soriano A, et al. (2013). Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) 2013: Unveiling the pathogenic, clinical and diagnostic aspects. *Journal of autoimmunity*, *47*, 1–16. Doi:10.1016/j.jaut.2013.10.004.

Petanjek Z, Judaš M, Šimic G, Rasin MR, et al. (2011). Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America 108*(32), 13281–13286. Doi:10.1073/pnas.1105108108.

Petersen SB, Gluud C. (2020). Was amorphous aluminium hydroxyphosphate sulfate adequately evaluated before authorisation in Europe? *BMJ Evidence-Based Medicine 26*, bmjebm-2020-111419. Doi: 10.1136/bmjebm-2020-111419.

Petrovsky N. (2015). Comparative Safety of Vaccine Adjuvants: A Summary of Current Evidence and Future Needs. *Drug Safety 38*(11),1059–74. Doi:10.1007/s40264-015-0350-4.

Petrovsky N, Aguilar JC. (2004). Vaccine adjuvants: Current state and future trends. *Immunology and Cell Biology, 82*(5), 488-496. Doi:10.1111/j.0818-9641.2004.01272.x.

Pilsner JR, Hu H, Wright RO, Kordas K, et al. (2010). Maternal MTHFR genotype and haplotype predict deficits in early cognitive development in a lead-exposed birth cohort in Mexico City. *The American Journal of Clinical Nutrition 92*(1), 226–234. Doi:10.3945/ajcn.2009.28839.

Purcell AE, Jeon OH, Zimmerman AW, Blue ME, et al. (2001). Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology 57*(9), 1618–1628. Doi:10.1212/wnl.57.9.1618.

Presumey J, Bialas AR, Carroll, MC (2017). Complement System in Neural Synapse Elimination in Development and Disease. *Advances in Immunology 135*, 53–79. Doi:10.1016/bs.ai.2017.06.004.

Qu Q, Zhang W, Wang J, Mai D, et al. (2022). Functional investigation of SLC1A2 variants associated with epilepsy. *Cell Death and Disease 13*(12). Doi:10.1038/s41419-022-05457-6.

Rahbar MH, Samms-Vaughan M, Ma J, Bressler J, et al. (2015). Interaction between *GSTT1* and *GSTP1* allele variants as a risk modulating-factor for autism spectrum disorders. *Research in Autism Spectrum Disorders 12*, 1–9. Doi:10.1016/j.rasd.2014.12.008.

Rahbar MH, Samms-Vaughan M, Zhao Y, Saroukhani S, et al. (2022). Interactions between Environmental Factors and Glutathione S-Transferase (GST) Genes with Respect to Detectable Blood Aluminum Concentrations in Jamaican Children. *Genes 13*(10), 1907. Doi:10.3390/genes13101907.

Rakic P. (1972). Mode of cell migration to the superficial layers of fetal monkey neocortex. *The Journal of Comparative Neurology 145*(1), 61–83. Doi:10.1002/cne.901450105.

Ramge P, Unger RE, Oltrogge JB, Zenker D, et al. (2000). Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human and bovine primary brain capillary endothelial cells. *The European Journal of Neuroscience 12*(6), 1931–1940. Doi:10.1046/j.1460-9568.2000.00078.x.

Rapaka RR. (2024). How do adjuvants enhance immune responses? *eLife 13*. Doi: 10.7554/eLife.101259.

Rempe R, Cramer S, Hüwel S, Galla HJ. (2011). Transport of Poly(n-butylcyano-acrylate) nanoparticles across the blood-brain barrier in vitro and their influence on barrier integrity. *Biochemical and Biophysical Research Communications 406*(1), 64-9. Doi:10.1016/j.bbrc.2011.01.110.

Rigor RR, Beard RS Jr, Litovka OP, Yuan SY. (2012). Interleukin-1β-induced barrier dysfunction is signaled through PKC-θ in human brain microvascular endothelium. *American Journal of Physiology-Cell Physiology 302*(10), C1513–C1522. Doi:10.1152/ajpcell.00371.2011.

Rodriguez JI, Kern, JK. (2011). Evidence of microglial activation in autism and its possible role in brain underconnectivity. *Neuron Glia Biology 7*(2-4), 205–213. Doi:10.1017/S1740925X12000142.

Rossignol DA, Frye RE. (2021). The Effectiveness of Cobalamin (B12) Treatment for Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. *Journal of Personalized Medicine 11*(8), 784. Doi:10.3390/jpm11080784.

Rossignol DA, Genuis SJ, Frye RE. (2014). Environmental toxicants and autism spectrum disorders: a systematic review. *Translational Psychiatry 4*(2), e360. Doi:10.1038/tp.2014.4.

Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. (2019). A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. *Journal of Psychiatric Research 115*. Doi:10.1016/j.jpsychires.2019.05.019.

Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, et al. (2014). The familial risk of autism. *JAMA 311*(17), 1770–1777. Doi:10.1001/jama.2014.4144.

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron 74*(4), 691–705. Doi:10.1016/j.neuron.2012.03.026.

Semple BD, Kossmann T, Morganti-Kossmann, MC. (2010). Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism 30*(3), 459–473. Doi:10.1038/jcbfm.2009.240.

Shaftel SS, Griffin WS, O'Banion MK. (2008). The role of interleukin-1 in neuroinflammation and Alzheimer disease: an evolving perspective. *Journal of Neuroinflammation 5*, 7. Doi:10.1186/1742-2094-5-7.

Shaw CA, Li Y, Tomljenovic L. (2013). Administration of aluminium to neonatal mice in vaccine-relevant amounts is associated with adverse long term neurological outcomes. *Journal of Inorganic Biochemistry 128*, 237–244. Doi:10.1016/j.jinorgbio.2013.07.022.

Shaw CA, Tomljenovic L. (2013). Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants, and autoimmunity. *Immunologic Research, 56*(2-3), 304–316. Doi:10.1007/s12026-013-8403-1.

Shaw KA, Williams S, Patrick ME, Valencia-Prado M, et al. (2025). Prevalence and Early Identification of Autism Spectrum Disorder Among Children Aged 4 and 8 Years - Autism and Developmental Disabilities Monitoring Network, 16 Sites, United States, 2022. *Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington, D.C.:2002), 74*(2), 1–22. Doi:10.15585/mmwr.ss7402a1.

Shoenfeld Y, Agmon-Levin N. (2011). "ASIA" – Autoimmune/inflammatory syndrome induced by adjuvants. *Journal of Autoimmunity 36*(1), 4–8. Doi:10.1016/j.jaut.2010.07.003.

Siddiqui MF, Elwell C, Johnson MH. (2016). Mitochondrial Dysfunction in Autism Spectrum Disorders. *Autism-Open Access 6*(5), 1000190. Doi:10.4172/2165-7890.1000190.

Silbereis JC, Pochareddy S, Zhu Y, Li M., et al. (2016). The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. *Neuron 89*(2), 248–268. Doi:10.1016/j.neuron.2015.12.008.

Singer HS, Morris CM, Williams PN, Yoon DY, et al. (2006). Antibrain antibodies in children with autism and their unaffected siblings. *Journal of Neuroimmunology 178*(1-2), 149–155. Doi:10.1016/j.jneuroim.2006.05.025.

Singh V, Kaur R, Kumari P, Pasricha C, Singh R. (2023). ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders. Clinica Chimica Acta; *International Journal of Clinical Chemistry 548*, 117487. Doi:10.1016/j.cca.2023.117487.

Singh VK, Lin SX, Newell E, Nelson C. (2002). Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. *Journal of Biomedical Science 9*(4), 359–364. Doi:10.1007/BF02256592.

Smith SE, Li J, Garbett K, Mirnics K, et al. (2007). Maternal immune activation alters fetal brain development through interleukin-6. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 27*(40), 10695–10702. Doi:10.1523/JNEUROSCI.2178-07.2007.

Sohal VS, Rubenstein JLR. (2019). Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. *Molecular Psychiatry 24*(9), 1248–1257. Doi:10.1038/s41380-019-0426-0.

Stephan AH, Barres BA, Stevens B. (2012). The complement system: an unexpected role in synaptic pruning during development and disease. *Annual Review of Neuroscience 35*, 369–389. Doi:10.1146/annurev-neuro-061010-113810.

Stevens B, Allen NJ, Vazquez LE, Howell GR, et al. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell 131*(6), 1164–1178. Doi:10.1016/j.cell.2007.10.036.

Stoner R, Chow ML, Boyle MP, Sunkin SM, et al. (2014). Patches of disorganization in the neocortex of children with autism. *The New England Journal of Medicine 370*(13), 1209–1219. Doi:10.1056/NEJMoa1307491.

Suzuki K, Matsuzaki H, Iwata K, Kameno Y, et al. (2011) Plasma Cytokine Profiles in Subjects with High-Functioning Autism Spectrum Disorders. *PLoS ONE 6*(5), e20470. Doi:10.1371/journal.pone.0020470.

Tan C, Frewer V, Cox G, Williams K, et al. (2021). Prevalence and Age of Onset of Regression in Children with Autism Spectrum Disorder: A Systematic Review and Meta-analytical Update. *Autism Research: Official Journal of the International Society for Autism Research, 14*(3), 582–598. Doi:10.1002/aur.2463.

Tang G, Gudsnuk K, Kuo SH, Cotrina ML, et al. (2014). Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron 83*(5), 1131–1143. Doi:10.1016/j.neuron.2014.07.040.

Tick B, Bolton P, Happé F, Rutter M, et al. (2016). Heritability of autism spectrum disorders: a meta-analysis of twin studies. *Journal of Child Psychology and Psychiatry, and Allied Disciplines 57*(5), 585–595. Doi:10.1111/jcpp.12499.

Tomljenovic L, Shaw CA. (2011). Aluminum vaccine adjuvants: are they safe? Current medicinal chemistry18(17), 2630–7. Doi:10.2174/092986711795933740.

Tomljenovic L, Shaw CA. (2011). Do aluminum vaccine adjuvants contribute to the rising prevalence of autism? *Journal of Inorganic Biochemistry 105*(11), 1489–1499. Doi:10.1016/j.jinorgbio.2011.08.008.

Tomljenovic L, Shaw CA. (2012). Mechanisms of aluminum adjuvant toxicity and autoimmunity in pediatric populations. *Lupus 21*(2), 223–30. Doi:10.1177/09612033114302.

Torres AR, Maciulis A, Stubbs EG, Cutler A, et al. (2002). The transmission disequilibrium test suggests that HLA-DR4 and DR13 are linked to autism spectrum disorder. *Human Immunology*, *63*(4), 311–316. Doi:10.1016/s0198-8859(02)00374-9.

Torres AR, Sweeten TL, Johnson RC. Odell D, et al. (2016). Common Genetic Variants Found in HLA and KIR Immune Genes in Autism Spectrum Disorder. *Frontiers in Neuroscience 10*, 463. Doi:10.3389/fnins.2016.00463.

Traiffort E, Kassoussi A, Zahaf A, Laouarem Y. (2020). Astrocytes and Microglia as Major Players of Myelin Production in Normal and Pathological Conditions. *Frontiers in Cellular Neuroscience 14*, 79. Doi:10.3389/fncel.2020.00079.

Treffert DA. (1970). Epidemiology of infantile autism. *Archives of General Psychiatry*, *22*(5), 431–438. Doi:10.1001/archpsyc.1970.01740290047006.

Uddin LQ, Supekar K, Menon V. (2013). Reconceptualizing functional brain connectivity in autism from a developmental perspective. *Frontiers in Human Neuroscience 7*, 458. Doi:10.3389/fnhum.2013.00458.

Vakilzadeh G, Martinez-Cerdeño V. (2023). Pathology and Astrocytes in Autism. Neuropsychiatric Disease and Treatment 19, 841–50. Doi:10.2147/NDT.S390053.

Vallese A, Cordone V, Ferrara F, Guiotto A, et al. (2024). NLRP3 inflammasome-mitochondrion loop in autism spectrum disorder. *Free Radical Biology and Medicine 225*, 581–94. Doi:10.1016/j.freeradbiomed.2024.10.297.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology*, *57*(1), 67–81. Doi:10.1002/ana.20315.

Vohra R, Madhavan S, Sambamoorthi U. (2016). Comorbidity prevalence, healthcare utilization, and expenditures of Medicaid enrolled adults with autism spectrum disorders. *Autism 21*(8), 995–1009. Doi:10.1177/1362361316665222.

Wang J, Ma B, Wang J, Zhang Z, Chen O. (2022). Global prevalence of autism spectrum disorder and its gastrointestinal symptoms: A systematic review and meta-analysis. *Frontiers in Psychiatry, 13*. Doi:10.3389/fpsyt.2022.963102.

Wang X, Wang J, Zhao X, Xie L, Yang R, Sun C, Tu J, Sun H. (2025). Drug-Dependent Enhancement of Blood-Brain Barrier Permeation by Polysorbate 80 Minor Components. *Pharmaceutics 17*(12), 1572. Doi:10.3390/pharmaceutics17121572.

Warren RP, Burger RA, Odell D, Torres AR, Warren WL. (1994). Decreased plasma concentrations of the C4B complement protein in autism. *Archives of Pediatrics & Adolescent Medicine 148*(2), 180–183. Doi:10.1001/archpedi.1994.02170020066011.

Watad A, Sharif K, Shoenfeld Y. (2017). The ASIA syndrome: Basic Concepts. *Mediterranean Journal of Rheumatology 28*(2), 64–9. Doi:10.31138/mjr.28.2.64.

Wei H, Zou H, Sheikh AM, Malik M, Dobkin C, Brown WT, Li X. (2011). IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. *Journal of Neuroinflammation 8*(52). Doi:10.1186/1742-2094-8-52.

Weisberg I, Tran P, Christensen B, Sibani S, et al. (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Molecular Genetics and Metabolism 64*(3), 169–172. Doi:10.1006/mgme.1998.2714.

Westacott LJ, Wilkinson LS. (2022). Complement dependent synaptic reorganisation during critical periods of brain development and risk for psychiatric disorder. *Frontiers in Neuroscience 16*, 840266. Doi:10.3389/fnins.2022.840266.

Wolff JJ, Gu H, Gerig G, Elison JT, et al. (2012). Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. *The American Journal of Psychiatry 169*(6), 589–600. Doi:10.1176/appi.ajp.2011.11091447.

Xiong Y, Chen J, Li Y. (2023). Microglia and astrocytes underlie neuroinflammation and synaptic susceptibility in autism spectrum disorder. *Frontiers in Neuroscience 17*. Doi:10.3389/fnins.2023.1125428.

Zhu X, Hao W, Liu Z, Song Y, Hao C, Wu S, et al. (2023). Aluminum induces neuroinflammation via P2X7 receptor activating NLRP3 inflammasome pathway. *Ecotoxicology and Environmental Safety 249*,114373–3. Doi:10.1016/j.ecoenv.2022.114373.