

Immunological Dysfunction in Autism Spectrum Disorder: A Potential Target for Therapy

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Keywords

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Abstract

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder with an unknown etiology and currently few effective therapies. Immune system alterations have been demonstrated in ASD, both in humans and via animal models; immune imbalance thus arises as a possible pathway for drug intervention. In this review, the studies were classified into 2 major groups: (1) clinical research whose authors classify therapies with primary anti-inflammatory and immunomodulatory actions, making use of: sulforaphane, celecoxib, lenalidomide, pentoxifylline, spironolactone, flavonoid luteolin, corticosteroids, oral immunoglobulin, intravenous immunoglobulin, cell therapy, dialyzable lymphocyte extracts, minocycline, and pioglitazone; and (2) other ASD ther-

apies already used or currently under study whose initial characteristics were neither anti-inflammatory nor immunomodulatory initially, but displayed a capacity for immunomodulation throughout the treatment: risperidone, vitamin D, omega-3, *Ginkgo biloba*, L-carnosine, N-acetylcysteine, and microbiome restoration. These studies used various data acquisition methodologies. Questions arose such the need for randomized and placebo-controlled studies with greater numbers of participants as well as the use of biomarkers to refine the treatment of autistic subjects.

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Introduction

Autism spectrum disorder (ASD) is a developmental disorder and its core symptoms are (1) deficits in social interaction, communication, and language, and (2) repetitive and stereotypical behaviors and/or a restricted rep-

ertoire of interests [1]. The diagnosis of autism (used here as synonymous with ASD throughout the text) has increased dramatically in the past decades [2, 3]. In 2018, the Centers for Disease Control and Prevention (CDC) has estimated the prevalence of ASD to be 16.8/1,000 (i.e., 1/59) in children 8 years of age, affecting 26.6/1,000 boys and 6.6/1,000 girls (i.e., a prevalence ratio of 4:1) [4]. This represents an increase of approximately 150% between 2000 and 2014 [4], making the disorder a public health problem [5, 6]. Studies in Asia, Europe, and North America report an average prevalence of ASD of around 1% [3].

Despite the increase in the number of cases, the treatments available today only partially improve some, but do not completely reverse all, of the symptoms of ASD [7–9]. Only 2 drugs, risperidone and aripiprazole, have been approved by the US FDA for the treatment of disruptive symptoms in ASD patients [8, 10–14]. In addition, factors such as (1) the condition of the individual, with many facets of daily functioning affected [15], (2) the substantial direct and indirect economic effects of treatments, and (3) how a subject's whole family suffers, reinforce the need for a continued search for effective interventions [16–20].

ASD is a complex neurodevelopmental disorder [3], and although a number of definitions and improvements have been made, its etiology remain unclear [2, 3, 21]. However, there is consensus in the autism research community that both genetic and environmental factors contribute to the symptoms, leaving no doubt of the multifactorial nature of ASD [2, 3, 21–23].

Immunological dysfunction has been a recognized feature in ASD for several decades and has been highlighted in recent revisions [2, 3, 8, 15, 21–26]. In 1976, a study found that 5 of 13 autistic children had undetectable antibody titers despite previous vaccination against Rubella while every control subject had detectable titers, making the first suggestion of a link between the immune system and ASD [26, 27]. Over the past few decades, studies on animal models and humans have shown evidence of alterations in central and peripheral immune system functioning in ASD, including stimulation of immune cells, generation of autoantibodies, cytokine/chemokine imbalance, and increased permeability of the blood-brain barrier [2, 3, 8, 15, 21–26]. Interestingly, many studies have also demonstrated a correlation between ASD status and cytokine levels and secretion [28–46], levels of immunoglobulins (IgM and IgG) [47], B lymphocyte antigen D8/17 expression [48], serum antineuronal antibodies [49–51], anti-ganglioside M1 antibodies [52], and maternal antibody status [51, 53]. These data support the

hypothesis that there is a subgroup of ASD individuals that has some form of immune system dysregulation, and that, at least in part, this dysregulation may contribute to the autistic phenotype [2, 3, 8, 15, 21–26].

Many researchers suggest the possibility that immune dysfunction in ASD may be a viable pathway for drug intervention, so that a subgroup of ASD individuals could benefit from immune-based therapies [2, 3, 8, 21–24, 26, 54–56]. This review aims to describe the drugs that act on the immune system that have been studied in patients with autism.

Methods

The studies in this review were obtained from a comprehensive search of PubMed using the terms and keywords “autism,” “autistic,” “autistic spectrum disorder,” “ASD,” “Rett,” “Asperger,” “Pervasive Developmental Disorder,” and “PDD” associated with the terms “treatment,” “inflammation,” “immunological drugs,” “immune,” “inflammation,” “inflammatory,” “anti-inflammatory,” “immunomodulation,” “immunology,” “immunological,” “neuroinflammation,” “neuroinflammatory,” “antibody,” “antibodies,” “immunoglobulin,” “lymphocyte,” “glial activation,” “cytokine,” “immunomodulatory,” “BDNF” “sulforaphane,” “pregnenolone,” “celecoxib,” “immunoglobulin,” “ACTH,” “lenalidomide,” “pentoxifylline,” “pioglitazone,” “spironolactone,” “corticosteroids,” “probiotics,” “luteolin,” “transplant,” “stem cells,” “cell therapy,” “autologous,” “vitamin D,” “risperidone,” “naloxone,” “minocycline,” and “lymphocyte extract.” The search included articles in English, Spanish, and Portuguese. Only studies conducted on humans were included.

Findings Relating Immunological Dysfunction in ASD and Potential Therapies

The inclusion of articles in this review was based on the classification criteria of the original articles which considered anti-inflammatory or immunomodulatory mechanisms of action in ASD therapies: (1) clinical research whose authors classified drugs with primary anti-inflammatory and immunomodulatory actions: sulforaphane, celecoxib, lenalidomide, pentoxifylline (PTX), spironolactone, flavonoid luteolin, corticosteroids, oral immunoglobulin, intravenous immunoglobulin (IVIG), cell therapy, dialyzable lymphocyte extracts, minocycline, and pioglitazone (Table 1) (the side effects of these thera-

Table 1. Summary of studies with immunomodulatory therapies in ASD

Therapy	Study	Dosage	Period	n (ASD)	Measures	Finding	Ref.
Sulfuraphane	placebo-controlled, double-blind, randomized clinical trial	50–150 µmol	4–18 weeks	40	ABC, SRS, CGI-I, S, CGI-I	Reduction in ABC, SRS, and CGI-I scores compared to placebo	[59]
Sulfuraphane	case series	n.r.	3 years	16	Descriptive	One of these families reported that the behavioral improvement was maintained even after the discontinuation of sulfuraphane use, and in 9 families, the patient is still taking SF with sustained improvement (follow-up of paper)	[60]
Celecoxib	placebo-controlled, double-blind, randomized clinical trial	200–300 mg	10 weeks	40	ABC	Improvements in irritability, social withdrawal, and stereotypic behavior subscales	[63]
Spirolonactone	case report	2 mg/kg	4 weeks	1	ABC, PPVT-III	Improvement in irritability, lethargy, stereotypy, hyperactivity, and inappropriate speech on ABC; increase in language gain in PPVT-III	[81]
Lenalidomide	open-label study	2.5 mg	12 weeks	7	CARS, ADOS, ROWPVT, CGI-I	Serum TNF-α reduction in 57%. Decreased symptoms of autism based on CARS scores, CGI expressive language, CGI receptive language at 6 weeks, and CGI expressive language at 12 weeks	[65]
PTX	placebo-controlled, double-blind, randomized clinical trial	400–600 mg	10 weeks	40	ABC, ESRS	Improvement in irritability, social withdrawal, stereotypic behavior, hyperactivity, and inappropriate speech ABC subscales	[75]
PTX	open-label study	150–600 mg	n.r.	23	Descriptive	"Remarkably effective" in 10 cases, "fairly effective" in 8, "slightly effective" in 3, and had "no effect" in 2	[70]
PTX	open-label study	n.r.	n.r.	30	Descriptive	Six patients (20%) showed a marked improvement in behavior and 14 (47%) showed a slight amelioration of symptoms	[71]
PTX	open-label study	200 mg	3 months	20	Seiken's Critical List for Autistic Children and patients' behavior in specific situations	35% of patients, improvement was observed in at least 2 of 3 assessments	[72]
PTX	open-label study	200 mg	4–10 months	2	Descriptive	Improvement in pronunciation of syllables and words	[73]
PTX	open-label study	10–15 mg/kg	3 months	20	EEG, "Checklist for Autistic Children," Parent-Child Relationship Test	EEG before and after PTX showed significant differences in 39% of patients. Improvement in "playing with a friend" and "personal communication in a psychopathological test"	[74]
Luteolin	case series	200 mg/10 kg	n.r.	37	Descriptive	Improvements in stool characteristics, allergic problems, eye contact, retention of learning, social interaction, attention to directions, and speech	[85]
Luteolin	open-label study	100 mg/10 kg	26 weeks	50	VABS, ABC, ATEC, CGI-I	Benefit in adaptive functioning (VABS scores) and in overall behavior (ABC)	[86]
Luteolin	case report	700 mg + 70 mg bid	12 months	1	ATEC	Improvement in motor stereotypies, hyperactivity, and cognitive abilities. Reduced enuresis	[88]
Luteolin	open-label study	100 mg	26 weeks	40	VABS	Improvement in age-equivalent communication, daily living skills, and social domains, and composite score	[87]
Prednisolone	case series	2 mg/kg	n.r.	44	CLSQ, FMAER, EEG	Changes in FMAER responses, improvement of language functions	[90]
Prednisolone	case report	0.5 mg/kg	1 year	1	REEL, Vineland, Rosetti, CARS	Improvement of social abilities, speech, and attention span	[91]
Prednisone	case report	0.5 mg/kg	28 weeks	1	WTSC-R	Improvement in language skills and behavior	[92]
Prednisolone	case series	2 mg/kg	30 + 48 months	2	Descriptive	Improvement in speech	[93]
Minocycline	placebo-controlled, double-blind, randomized clinical trial	100 mg	10 weeks	50	ABC, ESRS	Improvement in irritability and hyperactivity/noncompliance subscales	[126]
Minocycline	open-label study	1.4 mg/kg	6 months	11	CGI-I, VABS, DAS-II, NVDO	No clinical improvements	[125]
Progltazone	open-label study	30–60 mg	12–16 weeks	25	ABC	Improvement in hyperactivity, irritability, lethargy, and stereotypy subscales	[133]
Progltazone	placebo-controlled, double-blind, randomized, clinical trial	30 mg	10 weeks	40	ABC	Improvement in hyperactivity, irritability, and lethargy subscales	[134]
Oral human immunoglobulin	placebo-controlled, double-blind, randomized, clinical trial	140, 420, or 880 mg	12 weeks	125	MGIS	No different from placebo	[103]
Oral human immunoglobulin	open-label study	420 mg	8 weeks	12	GSI, ABC, CGI-S	Improvement of GI symptoms and behavior	[102]

Table 1 (continued)

Therapy	Study	Dosage	Period	n (ASD)	Measures	Finding	Ref.
IVIG	placebo-controlled, double-blind, randomized, clinical trial	400 mg/kg single dose	13 weeks	12	ABC, Symptom Checklist, CPRS	Amelioration of most ratings on ABC factors and symptom checklist scores	[105]
IVIG	open-label study	400 mg/kg monthly	6 months	5	Ritvo-Freeman Real-Life Rating Scale, C-YBOCS, CGI-AD, A-NIMH	No significant difference	[107]
IVIG	open-label study	1–6 doses of 154–375 mg/kg/dose	19 weeks	10	descriptive	Only 1 child had autism core symptoms relief	[106]
IVIG	open-label study	1 g/kg (10 doses)	31 weeks	14	CCC-2, SRS, ABC, CGI-S, CGI-I, ADOS, PPVT, CDI154, toll-like receptor-4, memory B cells, Foxp3, lymphocyte stimulation	Amelioration of speech and social interactions	[108]
CBMNC + UCMSC	open-label study	2 × 10 ⁶ CBMNC/kg + 10 ⁶ UCMSC/kg	4 infusions (1 every 5–7 days)	36	CARS, ABC, CGI	The combination was more beneficial than CBMNC alone, and both were more effective than only behavioral therapy	[110]
Fetal stem cells	open-label study	*	2 days	45	ATEC, ABC	Less agitation, and improvements in eye contact, appetite, and affect	[111]
Autologous BM-derived MNC	case report	56 × 10 ⁶ MNC intrathecally	a single infusion	1	CARS	CARS reduced from severely autistic to nonautistic, but the general impression on the clinical assessment showed mild autism	[112]
Embryonic stem cells	case series	**	4 sessions of 4–6 weeks	3	SPECT, descriptive	Improvements in eye coordination, balance, sensory processing, writing, and speaking	[116]
BM aspirate concentrate	open-label study	5 ml/kg	a single infusion	10	ISAA, WeeFIM	Improvements in ISAA	[114]
Autologous cord blood	open-label study	1–5 × 10 ⁷ cells/kg	a single infusion	25	VABS-II, CGI-S, CGI-I, PDDBI, EOWPVT-4 scales, EGT	Improved eye contact, improvement in all scales employed	[115]
Autologous BM-derived MNC	open-label study	8.19 × 10 ⁷ cells	a single infusion	32	ISAA, CGI-I, FIM, WeeFIM	Improvement in all scores	[113]
DLE	open-label study	150 IU	3 days every 6–12 weeks	40	SSSA	Amelioration and reversal of symptoms	[118]

Social Responsiveness Scale (SRS), Clinical Global Impression Severity (CGI-S) Scale, Clinical Global Impression Improvement (CGI-I) Scale, Penbody Picture Vocabulary Test III (PPVT-III), Receptive/Expressive One-Word Picture Vocabulary Tests (ROWPVT/ EOWPVT), Autism Diagnostic Observation Schedule (ADOS), Childhood Autism Rating Scale (CARS), Extrapyramidal Symptoms Rating Scale (ESRS), Seiken's Critical List for Autistic Children, Vineland Adaptive Behavior Scales, 2nd edition (VABS), Ability Scales 2nd Edition (DAS-II), Mullen Scales (NVQ), Modified Global Improvement Scale (MGIS), Gastrointestinal Severity Index (GSI), Autism Treatment Evaluation Checklist (ATEC), Clinical Language Status Questionnaire (CLSQ) Receptive-Expressive Emergent Language Test (REEL), Wechsler Intelligence Scale for Children revised (WISC-R), Children Yale-Brown Obsessive Compulsive Scale (C-YBOCS), Autism Modification of the NIMH Global Obsessive-Compulsive Scale (A-NIMH), Symptom Severity Score Average (SSSA), Indian Scale for Assessment of Autism (ISAA), Functional Independent Measure (FIM), Functional Independent Measure for Children (WeeFIM), IVIG, intravenous immunoglobulin; BM, bone marrow; PTX, pentoxifylline; GI, gastrointestinal; MNC, mononuclear cells; CBMNC, cord-blood mononuclear cells; UCMSC, umbilical cord-derived mesenchymal stem cells; DLE, dialyzable lymphocyte extract; n.r., not reported.
 * 1.6 mL of liver cell suspension with a cell count >30 × 10⁶ cells/mL at each transplantation; 2.29 ± 0.49 mL of brain progenitor cells with a cell count >8.70 × 10⁶/mL per transplantation.
 ** 0.25 mL of human embryonic stem cells (<4 million cells) i.m. once a day; 1 mL (<16 million cells) given twice a week i.v.; 1–5 mL every 7 days by supplemental routes; nasal spray administered twice a week.

Table 2. Adverse events

Therapy	Side effects	Ref.
Sulforaphane	Greater weight gain and a possible association with seizures	[59]
Lenalidomide	Rash and a transient drop of absolute neutrophil count	[65]
Pentoxifylline	Nausea, vomiting, low blood pressure, and headache	[70]
Pentoxifylline	Limited to the gastrointestinal tract	[72]
Luteolin	Increased irritability	[86]
Prednisolone	Cushing syndrome, irritability. Gastrointestinal bleeding and hypercalcemia (both self-limited)	[90]
Minocycline	Gastrointestinal and upper respiratory symptoms, pica, hematuria, weight gain, hyperactivity, urinary tract infection, otitis media, epistaxis, teeth staining, increased aggression, head-banging, sensitivity to light, appetite loss, and ritualistic behavior	[125]
Pioglitazone	Transitory and self-limited elevations in white blood count, glucose levels, and liver enzymes	[133]
Oral human immunoglobulin	Skin and subcutaneous tissue disorders	[103]
Oral human immunoglobulin	Vomiting, skin rash	[103]
Intravenous immunoglobulin	Mild headaches and infusion-site reactions	[108]
Mononuclear cells and mesenchymal stem cells	Self-limited low-grade fever	[110]
Autologous cord blood	Allergic reaction, skin changes, agitation, and childhood infections	[115]
Autologous bone marrow-derived mononuclear cells	Headache, nausea and vomiting, pain at the procedure site, and seizures	[113]

pies are described in Table 2); and (2) other therapies for ASD already used or currently under study whose initial characteristics were neither anti-inflammatory nor immunomodulatory, but displayed a potential immunomodulation capacity throughout the treatment: risperidone, vitamin D, omega-3, *Ginkgo biloba*, L-carnosine, N-acetylcysteine (NAC), and microbiome restoration.

Sulforaphane

Sulforaphane is an isothiocyanate derived from various *Brassica* vegetables (especially broccoli) [57] with anticancer effects [58] and a potential protective effect in neurodegenerative diseases [57]. This therapeutic effect is based on the upregulation of gene transcription related to cell mechanisms of protection against oxidative stress, inflammation, DNA-damaging electrophiles, and radiation [59].

A placebo-controlled, double-blind, randomized trial evaluated the effect of sulforaphane for up to 18 weeks in 40 male patients with ASD (26 sulforaphane-treated and

14 placebo-treated), at a dose of 50–150 μM per day. In the treatment group, the Aberrant Behavior Checklist (ABC) mean score differed at 4, 10, and 18 weeks from baseline, and at 18 weeks there was a decrease of 34% in the ABC score and 17% in the Social Responsiveness Scale (SRS) score. Significant improvement was observed in the ABC subscales: irritability, lethargy, stereotypy, and hyperactivity, and in the SRS subscales: awareness, communication, motivation, and mannerisms. After stopping sulforaphane treatment, both the ABC and SRS subscores tended to return to the baseline score. On the subscale analysis of Clinical Global Impression Improvement (CGI-I) scores at the 18th week, social interaction, aberrant behavior and verbal communication were much improved in sulforaphane users compared to in placebo-treated subjects. Sulforaphane treatment was safe and well-tolerated, but a higher weight gain over the period was observed compared with placebo. Two participants had single, unprovoked seizures; their relation to sulforaphane use could not be ruled out [59]. After 2 years, the

researchers reassessed the patients' progress. Among 16 patients, 1 family reported a maintained behavioral improvement even after the discontinuation of sulforaphane use, and in 9 families the patient was still taking sulforaphane with improvement [60].

Celecoxib

Celecoxib is a nonsteroidal anti-inflammatory drug that reduces the cytokine-induced activation of COX-2 and inhibits the NF- κ B pathway [61, 62]. Celecoxib was used as associated therapy in the ASD treatment in a randomized, double-blind, placebo-controlled study. Forty children were randomly allocated to a celecoxib + risperidone or placebo + risperidone group for 10 weeks. The dose was 200 mg/day for patients weighing <30 kg and 300 mg/day for patients >30 kg. At week 10, the risperidone + celecoxib group demonstrated significant improvements in irritability, social withdrawal, and stereotypic behavior evaluated as ABC subscales when compared to the risperidone + placebo group. No significant difference was observed between the 2 groups regarding extrapyramidal symptoms or other side effects [63].

Lenalidomide

Lenalidomide is an analog of thalidomide used in myelodysplastic syndromes and more recently for therapy after autologous hematopoietic stem cell (HSC) transplantation [64]. It has lower toxicity than thalidomide and displays a greater modulatory potency of tumor necrosis factor (TNF)- α and other immunomodulatory cytokines. Lenalidomide arises as a possible treatment in patients with elevated TNF- α in the serum or cerebrospinal fluid (CSF), interleukin (IL)-1, IL-6, or methyl CpG-binding protein (MeCP)-1 [65].

An open-label study was conducted on 7 autistic male subjects aged 6–12 years with elevated TNF- α in their CSF (>50 pg/mL) to evaluate the effects of daily treatment with lenalidomide (2.5 mg) for 12 weeks, with evaluation at weeks 6 and 12. At the end of the study, serum and CSF TNF- α were reduced by 57%, but this reduction did not reach statistical significance. Six children who completed the 6-week follow-up showed decreased symptoms of autism based on the Childhood Autism Rating Scale (CARS), and an improvement in CGI expressive and receptive language scores but not in One-Word Receptive Language Testing (ROWPVT) scores. At 12 weeks of follow-up, CGI expressive language remained improved from baseline, but CGI receptive language and CARS showed similar values. Differences in social skill were not significant compared to baseline. Three patients were withdrawn

from the study because of reactions to the drug which then disappeared [65].

Pentoxifylline

PTX is a xanthine analog known as a phosphodiesterase inhibitor [66] with modulatory effects on cytokines such TNF- α , IL-10, and interferon (IFN)- γ [67], and it was proposed as a potential treatment for autistic patients in the 1970s and 1980s. Gupta et al. [68, 69] described 5 case series in a 1996 review:

(1) Twenty-three ASD children were treated with PTX (150–600 mg/day orally). Within 1 month of therapy, the drug was considered “remarkably effective” in 10 cases, “fairly effective” in 8, “slightly effective” in 3, and had “no effect” in 2. Side effects in a small number of patients included nausea, vomiting, low blood pressure, and headache [70].

(2) Thirty ASD children were evaluable after receiving PTX (with no dosage or duration of treatment specified). Six patients (20%) showed a marked improvement in behavior and 14 (47%) had a slight amelioration of symptoms [71].

(3) Shimoide [72] treated 20 male ASD patients with PTX (most of them received 200 mg/day) for 3 months. They were evaluated by means of a mental development scale for young children, Seiken's Critical List for Autistic Children and regarding their behavior in specific situations. In 35%, improvement was observed in at least 2 of 3 assessments. Side effects were limited to the gastrointestinal (GI) tract.

(4) The results of treatment of 18 psychotic and 2 autistic (a boy of 5 years and a girl of 7 years) children with PTX (mostly 200 mg/day) for 4–10 months revealed “highly positive” improvements in behavior (12 children) and language (14 children). In the autistic children, an improvement in the pronunciation of syllables and words was noted. Side effects included excitation (3 patients) and disturbed sleep (2 patients), which disappeared without discontinuing PTX [73].

(5) PTX was administered to 20 children with early infantile autism at a dose of 10–15 mg/kg/day split into 2 doses, for 3 months, with the aim of evaluating electroencephalogram (EEG) activity changes via autoregressive analysis software (ARASIS). EEG before and after PTX administration showed significant differences in 7/18 (39%) patients. “Playing with a friend” and “personal communication in a psychopathological test” were improved [74].

A placebo-controlled, double-blind trial randomized 40 patients to either PTX + risperidone or placebo + ris-

peridone for 10 weeks, with evaluation at baseline and at weeks 2, 4, 6, 8, and 10. The dose of PTX was 400 mg/day for children weighing 10–40 kg and 600 mg for children >40 kg. The PTX + risperidone group demonstrated significantly greater improvement on 5 ABC subscales: irritability, social withdrawal, stereotypic behavior, hyperactivity, and inappropriate speech. No differences in extrapyramidal symptoms or side effects were observed [75].

Spironolactone

Spironolactone is a synthetic 17-lactone steroid, belonging to the “potassium-sparing diuretics” class of drugs and is known as a competitive aldosterone antagonist [76] with potent anti-inflammatory, immunologic, and hormone-modifying properties [77–80]. Currently, this drug has been used for acne, hirsutism, and precocious puberty, demonstrating safety and tolerability.

Bradstreet et al. [81] reported a case of a 12-year-old autistic boy with immune dysregulation, food allergies, and elevated testosterone levels. He demonstrated a significant reduction in both the severity and frequency of several aberrant symptoms during 4 weeks of daily spironolactone administration (2 mg/kg). Comparing the ABC scores before and after the spironolactone implementation, there was a 79% improvement in irritability, an 83% decrease in lethargy, a 60% reduction in stereotypy, a 72% reduction in hyperactivity, and a 67% decrease in inappropriate speech. There was a receptive language gain of 21 months, indicating an increase in vocabulary >1 SD at either age level as evaluated in the Peabody Picture Vocabulary Test III (PPVT-III).

Flavonoid Luteolin

Luteolin is a natural flavonoid with antioxidant and anti-inflammatory properties and is found in a myriad of plants [82]. Dietary sources of flavonoid luteolin include carrots, peppers, oregano, and olive oil [83]. In humans, luteolin has been demonstrated to inhibit the secretion of proinflammatory mediators from mast cells [84].

In 1 study, 37 ASD children (29 boys and 8 girls, age range 4–14 years) received a dietary flavonoid formulation (NeuroProteka) containing mainly luteolin (100 mg): 75.67% of the subjects had improvements in the color, shape, or smell of their stools; 51.35% got fewer “allergic problems”; 40.54% started to have better eye contact; 40.54% showed more retention of learned behavior; 37.83% had more social interaction; 32.43% improved their attention to directions; and 10.81% got better at speaking words or sentences. This study lacked any specific psychometric instrument or an independent assess-

ment by an external evaluator. The authors did not clarify treatment or follow-up duration [85].

Another open-label trial assessed the same dietary supplement (100 mg of luteolin) in 42 boys and 8 girls aged 4–10 years for a 26-week period. Primary outcomes were age-equivalent scores in the 3 Vineland Adaptive Behavior Scale (VABS) domains: communication, daily living skills, and social. Secondary outcomes were ABC, Autism Treatment Evaluation Checklist (ATEC), and CGI-I scores. There was a significant improvement in adaptive functioning (for all 3 above domains) measured by VABS age-equivalent scores, as well as in overall behavior indicated by the reduction in ABC subscale scores. There was a transient period (1–8 weeks) of increased irritability in 27 of the 50 participants [86].

In a 2015 work, 40 patients diagnosed with ASD received a luteolin-containing dietary formulation for 26 weeks, reducing autism symptoms and serum levels of TNF and IL-6. At baseline, IL-6 levels were elevated (with no statistical significance) and TNF serum levels were significantly higher in ASD patients. Interestingly, there were 2 subgroups of ASD subjects in this study, i.e., with low and high serum IL-6 and TNF levels. The latter group ($n = 10$) had a significant improvement in the VABS age-equivalent scores for all domains and in VABS composite score. At 26 weeks, patients had improved in several behavioral domains, equivalent to 9.73 months in the communication domain, 8.09 months in the social domain, and 6.64 months in the daily living skills domain [87].

In a recent study, an association of palmitoylethanolamide + luteolin (700 + 70 mg twice a day for 1 year) was given to a 10-year-old male autistic patient. The evaluation with the ATEC questionnaire showed a decrease in scores, showing improvement in behavioral results. The treatment reduced stereotypic behaviors, significantly improved the cognitive domain (observed by parents and teachers) and, curiously, decreased enuresis of the subject after 14 months of treatment [88].

Corticosteroids

Corticosteroids are anti-inflammatory drugs that inhibit the secretion of proinflammatory mediators, alter T cell activity, and may also interfere with microglial activation [89]. One retrospective study with oral prednisone (2 mg/kg/day for 9–12 months) treatment assessed ASD patients by means of the Clinical Language Status Questionnaire (CLSQ), Frequency Modulated Auditory Evoked Response (FMAER) testing, and EEG. For the treated group (20 participants), 9/14 electrode measurements of an FMAER 4-Hz response had a statistically significant

modification, but more importantly, this was found at the left posterior-inferior temporal electrode (TP9). On the other hand, no difference was seen in the control group (24 nontreated subjects). EEGs of the treated and control groups showed no differences. The steroid-treated group also had a significant increase in the mean CLSQ scores after the therapy [90].

Shenoy et al. [91] reported a response to steroid therapy in a case of ASD secondary to an autoimmune syndrome. The patient was treated with different regimens of oral prednisolone, with an average dose of 0.5 mg/kg daily. The ASD symptoms improved progressively during the course of 1 year after the corticosteroid therapy. In a similar study, a child with language and behavior regression at 22 months of age (diagnosed later with pervasive developmental disorder) received prednisone (2 mg/kg/day) for 28 weeks. The results observed were speech amelioration, better responsiveness to verbal communication, improved social relationships, and fewer motor stereotypies [92].

Mordekar et al. [93] reported on 2 patients diagnosed with childhood disintegrative disorder that were also treated with corticosteroids, showing a similar clinical improvement. On prednisone treatment (2 mg/kg daily), the first patient showed a recovery of speech at day 11 of therapy. The second subject, on the same dose for 1 week, had progressive speech improvement over the course of 48 months.

In the 1990s, several studies were conducted with ORG 2766, a synthetic analog of the adrenocorticotrophic hormone (4-9). However, ORG 2766 has no substantial steroidogenic activity and may exert its effects via an interaction with endogenous opioid systems [94–98]. Another open-label study using the neurosteroid pregnenolone found improvements in ASD patients; considering that this drug did not alter cortisol levels, the authors suggested that pregnenolone would act by modulating GABA_A receptors, altering the ratio of excitation/inhibition in key neural systems [99, 100].

Oral Immunoglobulin

Oral immunoglobulin, constituted predominantly of IgG, is a medication prepared from immunoglobulins obtained from human and bovine serum and bovine milk. Several studies demonstrate its survival to gastric exposure and a resistance to proteolytic digestion in the GI tract [101].

Schneider et al. [102] administered oral immunoglobulin in a pilot study with 12 male ASD subjects, considering the chronic GI disturbances in ASD due to an under-

lying deficiency in mucosal immunity. By the fourth-week assessment, 50% of subjects had improvements in GI symptoms.

Similarly, Handen et al. [103] tested oral human immunoglobulin efficacy for GI symptoms in ASD. One hundred and twenty-five ASD children were treated with different doses of immunoglobulin (140, 420, or 840 mg/day) or placebo. The primary outcome was measurement by a Modified Global Improvement Scale (MGIS), which showed no significant changes between the groups.

Intravenous Immunoglobulin

IVIG is a treatment that contains a pool of several molecules of IgG from the human plasma (>95% unmodified IgG) [104]. Although it is widely used, the mechanism of action of IVIG for the treatment of autoimmune disorders is still unclear [23].

A clinical trial with 12 male ASD children receiving a single dose of IVIG (0.4 g/kg) or placebo evaluated the subjects after 6 or 13 weeks of therapy. There were significant improvements in irritability, hyperactivity, inadequate eye contact, and inappropriate speech scores on the ABC, besides a better score for drowsiness. There were no side effects from the treatment [105].

In an open-label clinical trial, 5 children diagnosed with autism received monthly IVIG infusions at a dose of 400 mg/kg for 6 months. No improvements were noted and no side effects cited [106]. In another open-label trial, 10 ASD children with blood immunologic abnormality, such as an increased percentage of lymphocytes expressing the DR antigen, received 1–6 IVIG infusions (154–375 mg/kg). In 5 cases, no improvement was perceived; in 4 cases, the parents noticed a mild amelioration of attention and hyperactivity but no differences in the core symptoms of autism. One 5-year-old who received infusions of 375 mg/kg experienced remarkable relief of autistic symptoms (e.g., started to speak for the first time). There were no adverse effects [107].

A recent pilot study investigated the efficacy and tolerability of IVIG as 10 infusions of 1 g/kg every 21±7 days in 14 autistic patients with an immunological imbalance (e.g., T or B cell dysfunction or recurrent infections). The amelioration of behavioral symptoms was measured by means of SRS, PPVT, Children's Communication Checklist 2 (CCC-2), ABC, and Autism Diagnostic Observation Scale (ADOS) scores. For the SRS, there was a significant improvement in overall score, autistic mannerisms, social cognition, and social motivation. On the CCC-2, advances in the speech and semantics domains were reported. The ABC scores did not change. On the other hand, the

ADOS scores changed significantly regarding stereotyped behaviors and restricted interests, communication and social interaction, and reciprocal social interaction. In relation to immunological markers after treatment, there were alterations in TNF- α and IL-1 β levels. Adverse events with IVIG included mild headaches and infusion-site reactions [108].

Cell Therapy

Conceptually, cellular therapy with HSC (cord-derived and bone marrow-derived) may help the inflammation balance through immune regulation [109].

An open trial studied 36 ASD patients treated with placebo, cord-blood mononuclear cells (CBMNC), or umbilical cord-derived mesenchymal stem cells (UCMSC) + CBMNC. The only adverse event reported during the follow-up period was a self-limited low-grade fever in 5 subjects in the combination treatment group. Similarly, no significant hepatic, renal, hematological, or metabolic abnormalities occurred after therapy. Both intervention groups showed significant improvement in CARS and total ABC scores at 24 weeks in comparison to baseline, with the combination group presenting better results than the CBMNC-only group. Statistically significant differences were also shown on CGI evaluation in the 2 treatment groups compared to controls at 24 weeks [110].

The efficacy and safety of fetal stem cell therapy was verified in 45 ASD subjects by ATEC and ABC evaluation, laboratory tests, and clinical examination. After 6 months of therapy, B cell counts decreased significantly, while CD3 and CD4 counts were increased after 12 months. A significant lowering of ATEC overall score was seen 12 months after therapy, and in total ABC scores after 6 and 12 months. No side effects were observed [111].

Initially, Sharma et al. [112] used autologous bone marrow mononuclear cells (BMMNC) intrathecally in a 14-year-old boy, noting symptomatic improvement, with a change in CARS from severely autistic to nonautistic and enhanced brain function on positron emission tomography-computed tomography (PET-CT).

Subsequently, an open-label proof-of-concept study used intrathecal BMMNC transplantation in association with occupational, speech, and psychological therapies in 32 ASD patients. There was a significant difference between the pre- and posttreatment CGI-I scores and in total Indian Scale for Assessment of Autism (ISAA) scores. All ISAA domains also showed significant decreases. The Functional Independence Measure (FIM) and FIM for Children (WeeFIM) scores showed no changes. PET-CT detected changes in glucose metabolism in the form of

FDG uptake in different cerebral regions. Seizures and minor adverse events, such as headache, nausea, vomiting, and pain related to the procedure were reported [113].

An open-label trial tested the effects of intrathecal administration of autologous bone marrow concentrate (BMAC) cell therapy in 10 subjects with autism. Mean ISAA score of the patients decreased (i.e., improved) by 8% in the first 3 months after BMAC injection, and further diminished by 6% from 3 to 6 months. Changes in WeeFIM score did not reach statistical significance. The patients showed no adverse reactions [114].

Another open-label clinical trial tested the safety and feasibility of administering an autologous cord-blood infusion to 25 ASD children. Significant improvements in behavior were found across a wide range of outcome measures: the VABS-II socialization domain, the CGI-I, the Pervasive Developmental Disorder Behavior Inventory (PDDBI), and the Expressive One-Word Picture Vocabulary Test 4 (EOWPVT). Most of the observed behavioral changes occurred during the first 6 months and were sustained between 6 and 12 months. A higher nonverbal IQ was associated with greater improvements. The Eye-Gaze Tracking showed a 20% increase that the children would gaze at an actress' eyes. Mild adverse events were seen: allergic reactions to infusion, skin changes, agitation, and common childhood infections [115].

Lastly, a case series report described improvement in 3 children with autism after an infusion of human embryonic stem cells. Better communication, attention to gaze, and cognitive performance were observed, besides improvements in brain blood perfusion reflected in the PET-CT. No side effects were noted [116].

Dialyzable Lymphocyte Extract

Dialyzable lymphocyte extracts are complexes of low-molecular-weight substances with the capacity for cell-mediated immunity transfer, mainly due to the action of small peptides called "transfer factors" [117]. A study published in 1996 reported a follow-up of forty 5-year-olds with classical infantile autism and pseudoautism after three and a half years of dialyzable lymphocyte extract therapy. Twenty-eight patients showed improvement and 10 had remission of autistic symptoms. The article did not clarify the criteria used to define autism and pseudoautism [118].

Minocycline

Minocycline is a tetracycline antibiotic with potent anti-inflammatory and neuroprotective effects [119, 120]. The exact immunomodulatory mechanism is not clear

[121]. Minocycline decreases microglial activation, modulates pathways involved in neuroinflammation such as cytokine and chemokine networks (e.g., IL-6, IL-1 β , and TNF- α), and reduces the activity of some metalloproteinases [122, 123]. Clinical trials on patients with X-fragile syndrome have shown minocycline to be effective and safe in improving behavioral function [124, 125].

An open-label pilot trial was assessed with 11 ASD children treated with minocycline (1.4 mg/kg/day) + vitamin B₆ (0.6 mg/kg, to mitigate the potential for vestibular side effects) [126]. Clinical improvements were negligible, with the CGI Severity (CGI-S) score remaining stable and only 2 of 10 children demonstrating a “minimal improvement” on the CGI-I. VABS composite scores also showed little or no change. Adverse events reported by parents included GI and upper respiratory symptoms, hematuria, weight gain, pica, hyperactivity, urinary tract infection, otitis media, epistaxis, teeth staining, increased aggression, head-banging, sensitivity to lights, increased appetite, and ritualistic behavior. Laboratory assays demonstrated significant changes in the expression profile of brain-derived neurotrophic factor (BDNF) and hepatic growth factor (HGF) in the CSF and serum. Among the evaluated cytokines, only IL-8 was significantly reduced in the serum after treatment, with no changes in noted in the CSF. No significant pre- and posttreatment changes were seen in the profiles of plasma metalloproteinases.

Ghaleiha et al. [127] conducted a randomized, double-blind, placebo-controlled trial with 50 ASD children that received minocycline (50 mg twice a day) + risperidone or placebo + risperidone for 10 weeks. The risperidone dose was 1 mg/day for patients weighing <20 kg and 2 mg/day for those weighing \geq 20 kg. A significantly greater score reduction in the irritability and hyperactivity/noncompliance subscales of the ABC occurred in the minocycline group when compared with the placebo group at week 10.

Pioglitazone

Pioglitazone belongs to the thiazolidinedione group and works via peroxisome proliferator-activated receptor (PPAR)- γ [128]. This drug is frequently used as an anti-diabetic agent and offers additional cardiovascular protection and lipid profile improvement [129]. PPAR ligands also have anti-inflammatory effects, downregulating or upregulating different components of the inflammatory response [130]. Thiazolidinediones have been tested in inflammatory disorders such as psoriasis [130] and asthma [131]. Pioglitazone effects on neuropsychiatric diseases have also been studied [132].

An open-label study evaluated the use of pioglitazone in 25 ASD subjects (age range 3–17 years) for 12–16 weeks with daily doses of 30 mg (for the age range 3–5 years) or 60 mg (age 6–17 years). Analysis revealed a significant decrease in 4 ABC subscales, hyperactivity, irritability, lethargy, and stereotypy, after the administration of pioglitazone; 76% of the patients showed a 50% reduction in at least 1 subscale and there was a tendency for younger participants to benefit more from this drug. Transitory and self-limited elevations in white blood count, glucose level, and liver enzymes were noted [133].

Ghaleiha et al. [134] conducted a double-blind placebo-controlled trial of pioglitazone (30 mg/day) as adjunctive treatment to risperidone during 10 weeks in 44 patients with ASD (aged 4–12 years). The pioglitazone group showed a significantly greater improvement in 3 ABC subscales, hyperactivity, irritability, and lethargy. The frequency of adverse effects was similar in the intervention and control groups.

Risperidone

Risperidone, an atypical antipsychotic, is widely used for the treatment of children with autism, and reduces the major behavioral disturbances such as irritability, aggression, and anxiety [135]. Studies suggest that risperidone has immunoregulatory effects, since it ameliorates several immunological changes (e.g., the expression of inflammatory cytokines, IL-1 β , and TNF- α) [136–138]. Treatment has been associated with a decrease in serum levels of eotaxin and monocyte chemoattractant protein (MCP)-1. In addition, mean values of IL-5 were significantly higher in the group that responded to risperidone than in the nonresponders [139]. On the other hand, a previous study on ASD subjects found no changes in serum inflammatory markers after risperidone use despite clinical improvements [140]. Therefore, the association between immunological changes and clinical response is a possibility that should be further investigated.

Supplements

Vitamin D

Vitamin D deficiency has been implicated as a potential environmental factor linked to some autoimmune disorders and it plays an immunomodulatory role [141, 142]. Several studies have found lower levels of vitamin D in ASD children than in healthy controls [143–145]. Mostafa and Al-Ayadhi [141] reported that vitamin D deficiency could be involved in autoantibody production in patients with autism, and Cannell [146] suggested that vitamin D may reduce the severity of autism symptoms

by its anti-inflammatory activity. A case report [147], along with open and controlled trials [142, 148], observed an ASD symptom scores reduction after vitamin D₃ supplementation. However, recent placebo-controlled clinical trials have found divergent results following vitamin D₃ supplementation for ASD [149, 150].

Omega-3

Omega-3 fatty acids have anti-inflammatory and immunomodulatory properties [151–153]. Supplementation with omega-3 decreases NF- κ B, IL-12, and IL-13 gene expression [154], and levels of macrophage inflammatory protein (MIP)-2, IL-6 [155], IL-17A [156], and TNF- α [155–158]. Despite being widely used as an alternative practice in children with ASD [159, 160], omega-3 supplementation cannot be recommended based on the evidence that is currently available [151, 160–164].

G. biloba (Ginkgoaceae) is an ancient Chinese tree with anti-inflammatory effects [165] and is one of the most globally consumed phytomedicines [166]. The effect of *G. biloba* on ASD has been studied in an open trial [167] and in a double-blind, placebo-controlled trial as an adjuvant therapy to risperidone [168], with 3 and 47 patients, respectively. It appeared to be safe and well-tolerated but did not demonstrate efficacy for autistic symptoms.

L-Carnosine

L-Carnosine is a dipeptide of the β -alanine and l-histidine amino acids. It is easily absorbed in the digestive tract, penetrates the blood-brain barrier, and has high bioavailability and membrane-stabilizing action [169]. Carnosine is a known antioxidant and neuroprotective agent [170, 171] and reduces inflammation and neurodegeneration by lowering TNF- α and nitric oxide synthesis [172]. The beneficial effects of L-carnosine on ASD symptoms could be attributed to immunomodulatory, antioxidant, glutamatergic, and NMDA- and GABA-modulatory activities [173].

Two randomized, placebo-controlled trials studied L-carnosine in ASD children at a dose of 800 mg/day. Chez et al. [174] evaluated 31 children for 8 weeks and found changes in multiple domains in the group that was given carnosine (receptive speech, social attention, and behavior). Hajizadeh-Zaker et al. [173] used L-carnosine + risperidone or placebo + risperidone for 10 weeks in 50 children. They found improvement in the hyperactivity/non-compliance ABC subscale in the L-carnosine group. A recent placebo-controlled clinical trial using 500 mg of L-carnosine for 2 months observed positive reductions in

the scores for sleep duration, parasomnia, and total sleep disorders, but no behavioral changes in ASD children [175].

N-acetylcysteine

NAC is an orally bioavailable prodrug of cysteine known for its use in acetaminophene overdose [176]. With anti-inflammatory properties through several cellular processes [177], NAC is a glutathione precursor and direct antioxidant, and hence inhibits upstream events leading to the activation of NF- κ B activation and other proinflammatory cytokines [177]. NAC also acts by inhibiting the inflammatory cytokines TNF- α , IL-1 β , and IL-6, at the protein and microRNA levels, in lipopolysaccharide (LPS)-activated macrophage cell lines [178, 179].

Marler et al. [180] reported a case of a 4-year-old boy who showed improvement in refractory self-injury after NAC use, with the symptoms returning after stopping the medication and improving again after its restart. Other case report also describes improvements in severe aggression in adolescents after the use of NAC in association with quetiapine [181]. Ghanizadeh and Derakhshan [182] reported gains in social interaction, communication, hyperactivity, limited interests, and aggressive behaviors in an 8-year-old boy after NAC use. A randomized, placebo-controlled trial by Hardan et al. [183] demonstrated a significant reduction in irritability symptoms in 29 children with ASD. Additionally, 2 randomized, placebo-controlled trials of NAC in conjunction with risperidone for the treatment of irritability in patients with ASD (with 50 and 40 participants, respectively) showed a significant reduction in the ABC irritability subscale score in the NAC-treated groups [184, 185]. More recently, 2 other randomized, placebo-controlled trials found no benefit of NAC in children with autism [186, 187].

Microbiome Restoration

There is an elevated prevalence of GI disturbances in patients with ASD, such as fluctuations in bowel habits, chronic abdominal pain, and flatulence. Interestingly, untreated GI symptoms may worsen core behavioral symptoms. A proposed mechanism for these phenomena is a disruption in the “gut-brain axis,” a communication between the enteric nervous system and the CNS. It has been suggested that the gut-brain axis conveys fundamental signals for neurodevelopment, and that its malfunctioning could be associated with the onset of neuropsychiatric disorders such as ASD [188].

Gut microbiota may be the agents of disruption in the gut-brain axis through indirect effects on the innate im-

mune system. Changes in gut microbiome are thought to be related to the decreased integrity of the intestinal barrier, leading to the augmentation of toxin absorption e.g., LPS. LPS can act on hepatocytes, inducing the secretion of TNF- α which, in turn, stimulates the production of proinflammatory cytokines systemically, ultimately leading to brain microglial activation [189]. Hence, based on these statements, researchers suggest that strategies of restoring the physiological gut microbiome, such as probiotic supplementation and fecal microbiota transplant, might be able to improve GI and behavioral symptoms in ASD [190]. In the year 2000, Sandler et al. [191] described a series of autistic patients with regressive autistic traits and chronic diarrhea. Treatment with the antibiotic vancomycin was successful in providing short-term improvement in 8 of 10 children, but the gains did not endure [24].

A research paper by Kałużna-Czaplińska and Błaszczuk [192] presented the results of dietary supplementation with probiotics which decreased a fungal urinary biomarker (D-arabinitol) and improved ability of concentration and carrying out others in subjects diagnosed with ASD. Similarly, an open-label study published in 2017 tested the effects of fecal microbiota transfer on autistic subjects; the authors reported an improvement of GI symptoms of as high as 80% and a significant amelioration of behavioral disturbances [193]. Lastly, a clinical trial randomized infant participants to receive either placebo or a probiotic dietary supplement during the first 6 months of life. While 6 of 35 subjects in the control group developed either attention deficit and hyperactivity disorder or Asperger syndrome later in life, none of the probiotics group had this outcome [194].

Discussion

ASD is a complex neurodevelopmental disorder [3], and studies of animal models and in humans demonstrate alterations in the immune system across the spectrum [2, 3, 8, 15, 21–26]. Several studies correlate ASD symptoms and immune status [28–48, 50–53], supporting the hypothesis that there is a subgroup of ASD individuals who could benefit from immune-based therapies [2, 3, 8, 21–24, 26, 54–56].

The ability of anti-inflammation/immunological modulation has been proposed in drugs routinely used for neurological and psychiatric diseases aside from their original mechanism of action. Aripiprazole, an antipsychotic approved by the FDA for use in ASD [14], has dis-

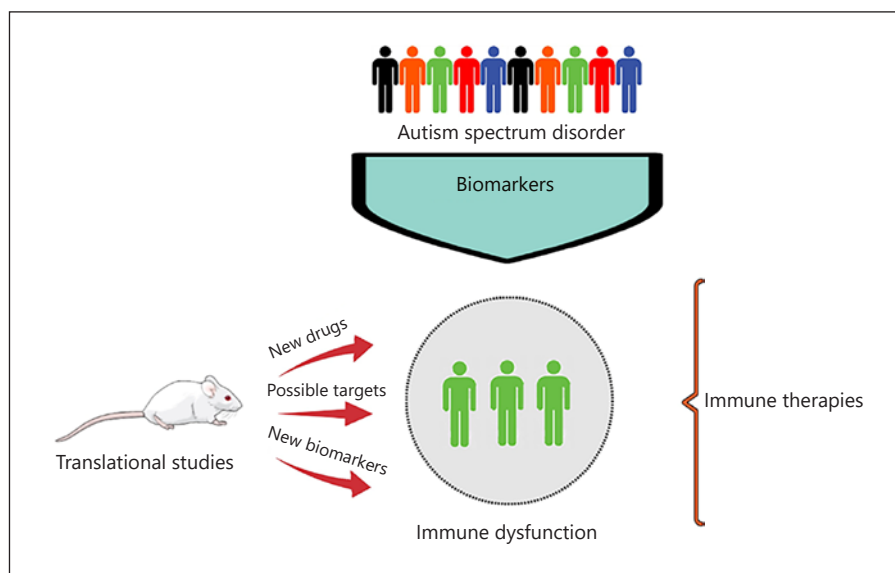
played immunological properties in *in vitro* experiments [195] and it limits inflammatory processes by enhancing the anti-inflammatory signaling in schizophrenia [196]. Valproic acid, a short-chain fatty acid used as a mood stabilizer and antiepileptic drug [197], may have anti-inflammatory as well as antioxidative effects [198–200].

The potential role of serotonergic transmission and NMDA receptors in the modulation of neuroinflammation has also been studied and could open new therapeutic perspectives to address the behavioral deficits in ASD [24, 54]. Buspirone [95, 201–203], fluoxetine [204–208], escitalopram [209, 210], citalopram [211], sertraline [212–214], D cycloserine [186, 215–218], amantadine [219], and memantine [220–222] have already been used in clinical research into autism. Finally, naltrexone, a potent opiate antagonist tested in ASD for improving behavioral changes [223–239], could also possess immunomodulatory action [240].

Currently, no available therapy can reverse the core symptoms of autism. The use of immune-based therapies may be a possible treatment route for some individuals, but only a few studies aimed at immunomodulation have been performed on ASD patients. These studies cover an extensive range of drug classes, including plant products, dietary supplements, probiotics, antibiotics, anti-inflammatory agents, immunomodulators, cell therapies, and even curious substances such as camel milk [241, 242]. Most are case reports or open trials with no control group. Only a few are placebo-controlled and have a sufficient number of participants to generate reliable findings. The heterogeneity of the study populations (regarding age, ethnic origin, and the severity of symptoms), the disparity in ASD diagnostic criteria, and the variety of psychological evaluation tools make it difficult to compare findings. In addition, many of the reports show important methodological flaws. Conflicting results in different studies on the same therapy are also frequent, and ASD symptoms showing improvement vary considerably. Lastly, there are only a few publications on each intervention, so further studies are needed to obtain consistent data.

It is possible that only some patients in the studies had immune dysfunction contributing to their etiology. Consequently, positive responses to a specific therapy in these patients could be overlooked when they are analyzed in a pool with nonresponders. The development of potential biomarkers of immune dysregulation and their correlation with phenotypic variability should increase the possibility of preselecting patients with a greater potential for improvement with immunomodulatory intervention. In-

Fig. 1. Immunological endophenotypes in autism. The identification of specific markers related to different ASD subgroups may assist in reducing the heterogeneity of participants in clinical trials. In addition, animal model trials enable the translational study of new drugs, possible targets, and biomarkers.



flammatory markers, including miRNA targets, appear to have potential for the development of individualized treatment strategies in the future [3, 243–247] (Fig. 1).

Despite the discrepancies in results and the methodological limitations found in the studies cited in this review, the possible existence of a subgroup of ASD patients who are true responders to immunological treatment cannot be excluded. Studies to investigate this possibility must be carried out, and the effectiveness of therapies previously tested on humans or in animal models should be evaluated. Drugs with anti-inflammatory potential such as donepezil, resveratrol, and fingolimod have demonstrated positive findings in animal models of ASD [248–255], pointing to promising therapeutic potential, so we would encourage further research to be undertaken.

Conclusions

The development of effective therapies in ASD is urgent. Despite immune dysfunction being a possible pathway for drug intervention in a subgroup of ASD individuals, few studies aiming an immunomodulatory action were performed. Besides, the use of potential biomarkers as screening tools may enable the selection of specific subjects for clinical trials and, in the future, determine which patients could benefit from a particular therapy. Most of the studies included in this review are not placebo-controlled and use different methodologies, making the in-

terpretation of different results a challenge. Studies with a greater number of participants, randomized and placebo-controlled, either with interventions already described in humans or with new therapies from translational studies in animal models are needed.

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