



# Immunoregulatory and/or Anti-inflammatory Agents for the Management of Core and Associated Symptoms in Individuals with Autism Spectrum Disorder: A Narrative Review of Randomized, Placebo-Controlled Trials

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Accepted: 13 February 2023 / Published online: 13 March 2023  
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## Abstract

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition with a so far poorly understood underlying pathogenesis, and few effective therapies for core symptoms. Accumulating evidence supports an association between ASD and immune/inflammatory processes, arising as a possible pathway for new drug intervention. However, current literature on the efficacy of immunoregulatory/anti-inflammatory interventions on ASD symptoms is still limited. The aim of this narrative review was to summarize and discuss the latest evidence on the use of immunoregulatory and/or anti-inflammatory agents for the management of this condition. During the last 10 years, several randomized, placebo-controlled trials on the effectiveness of (add-on) treatment with prednisolone, pregnenolone, celecoxib, minocycline, *N*-acetylcysteine (NAC), sulforaphane (SFN), and/or omega-3 fatty acids have been performed. Overall, a beneficial effect of prednisolone, pregnenolone, celecoxib, and/or omega-3 fatty acids on several core symptoms, such as *stereotyped behavior*, was found. (Add-on) treatment with prednisolone, pregnenolone, celecoxib, minocycline, NAC, SFN, and/or omega-3 fatty acids was also associated with a significantly higher improvement in other symptoms, such as *irritability*, *hyperactivity*, and/or *lethargy* when compared with placebo. The mechanisms by which these agents exert their action and improve symptoms of ASD are not fully understood. Interestingly, studies have suggested that all these agents may suppress microglial/monocyte proinflammatory activation and also restore several immune cell imbalances (e.g., T regulatory/T helper-17 cell imbalances), decreasing the levels of proinflammatory cytokines, such as interleukin (IL)-6 and/or IL-17A, both in the blood and in the brain of individuals with ASD. Although encouraging, the performance of larger randomized placebo-controlled trials, including more homogeneous populations, dosages, and longer periods of follow-up, are urgently needed in order to confirm the findings and to provide stronger evidence.

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## 1 Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by early-appearing (and persistent) deficits in social interaction and communication, sensory-motor problems, and by repetitive and/or stereotyped behaviors [1]. With a current global prevalence of about 1%, ASD is associated with a high community, and individual burden [2, 3]. However, and despite many efforts and significant advances in our understanding of the neurobiology of this condition, its exact etiology remains elusive [4]. As a consequence, pharmacological options for the management of ASD are still limited, and almost exclusively aimed at targeting associated (e.g., irritability, hyperactivity), but not core symptoms [5, 6].

### Key Points

Individuals with autism spectrum disorder (ASD) show immune and inflammatory abnormalities, including increased blood levels of (activated) monocytes, and abnormal blood levels of lymphocyte subpopulations, across the lifespan.

Individuals with ASD are also characterized by a proinflammatory activation of microglia in the brain, across the lifespan.

Immune system abnormalities found in individuals with ASD are a possible pathway for new drug intervention.

(Add-on) treatment with immunoregulatory/anti-inflammatory agents, such as prednisolone, pregnenolone, celecoxib, minocycline, *N*-acetylcysteine, sulforaphane, and/or omega-3 fatty acids may be beneficial for the management of core (e.g., *stereotyped behavior*) and associated (e.g., *irritability, hyperactivity, lethargy*) symptoms in individuals with ASD.

During the last decades, an increasing body of evidence has suggested the involvement of the immune system in the pathophysiology of this condition. Genome-wide association studies have demonstrated that variations in several genes that encode proteins involved in the inflammatory response (e.g., human leukocyte antigen [*HLA*] genes), may increase the risk of developing ASD [7]. In addition, maternal immune activation, and/or suffering from an infection during pregnancy also increase the risk of developing ASD in the offspring [8, 9]. Individuals with ASD often display abnormal immune responses [10, 11], and are also characterized by a higher incidence of comorbid immune-mediated conditions, such as diabetes mellitus [12], psoriasis [13], autoimmune thyroiditis [14], and/or allergy [15]. However, no consistent and/or specific immunological mechanism has emerged, so far.

Contrary to previous beliefs, the central nervous system (CNS) is not immune-privileged, and immune mediators, such as microglial cells, and/or T lymphocytes are normally present in the brain [16]. These cells are crucial for a proper brain development and function. For example, microglial cells participate in programmed neuronal death and promotion of synaptogenesis, and are able to strip excess synapses from developing neurons, allowing the integration of functional neuronal circuits [17–19] (Fig. 1). In addition, and while T-helper regulatory cells (Tregs) promote myelination [20], T-helper 17 cells (Th17) promote demyelination [21]. It has been therefore hypothesized that abnormal levels, or an abnormal function of microglia and of several immune

cells may lead to an abnormal development of the brain, and thus, predispose to neurodevelopmental conditions, such as ASD (Fig. 1). In support of this idea, microglial dysfunction has been related to core ASD symptoms, such as stereotyped behaviors, in a mouse model of obsessive-compulsive disorder [22]. Other reports have demonstrated the existence of a positive association between the severity of ASD symptoms and the levels of Th17 cells (and/or related cytokines, such as interleukin [IL]-17A) [23, 24].

Interestingly, an increasing body of evidence has suggested the existence of a systemically and chronically activation of microglia and of the monocyte system in individuals with ASD, across the lifespan [25]. Supporting this idea, a significantly higher microglial cell density, and/or a significantly higher expression of microglial cell activation markers, such as CD45, have been repeatedly found in the brain of individuals with ASD [26–28]. Moreover, elevated blood levels of monocytes [29–35], and an increased expression of different monocyte activation markers (e.g., CD96, HLA-DR) have been also demonstrated [36, 37]. Abnormal levels of several lymphocyte subpopulations, including natural killer (NK), B, CD8<sup>+</sup> T cytotoxic (Tc), and CD4<sup>+</sup> T helper (Th) cells have been demonstrated, both in the brain and in the blood of individuals with ASD [25, 29, 38–41]. Among the different Th cell subpopulations, an increase in Th17 cell levels, and a decrease in Treg levels, have been suggested [42, 43]. These findings are consistent with the cytokine profile that has been described in individuals with ASD, with subjects with ASD showing increased levels of proinflammatory cytokines and/or chemokines (e.g., interferon [IFN]- $\gamma$ , IL-1 $\beta$ , IL-6, IL-12p40, IL-17A, IL-31, tumor necrosis factor [TNF]- $\alpha$ , chemokine C-C-motif ligand [CCL]-2), and decreased levels of anti-inflammatory cytokines (e.g., IL-10) in their blood, and/or cerebrospinal fluid [44–48].

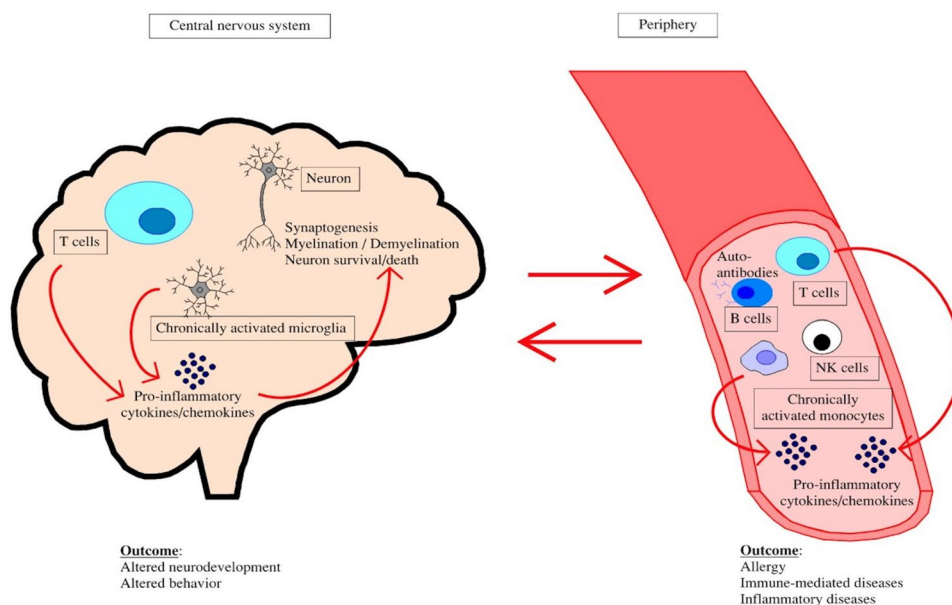
Current evidence on the role of the immune system in the pathophysiology of ASD has therefore increased the interest on the potential use of immunoregulatory and/or anti-inflammatory agents for improving core and associated symptoms in, at least, a subgroup of individuals with ASD. The aim of this narrative review was to investigate the existing evidence on the effectiveness of these agents for the management of ASD.

## 2 Literature Search

### 2.1 Search Strategy

The PubMed, SCOPUS, and World of Knowledge electronic databases were independently screened by two authors (GAH, LG) for relevant articles published between 1 January 1994, and 5 April 2022. The following search syntax was used for the PubMed database search, and adapted

**Fig. 1** Immune system abnormalities in ASD



according to the different database index terms: (autism OR autistic OR autism spectrum disorder OR ASD OR pervasive OR pervasive developmental disorder OR pervasive developmental disorders OR Asperger OR Asperger's) AND (celecoxib OR aspirin OR minocycline OR anti-inflammatory OR NAC OR *N*-acetylcysteine OR NSAID OR ACTH OR prednisone OR prednisolone OR corticosteroids OR hydrocortisone OR methylprednisolone OR dexamethasone OR cortisone OR sulforaphane OR omega-3 OR fatty acids OR immunomodulatory).

## 2.2 Eligibility Criteria

Inclusion and exclusion criteria were discussed and approved by all authors; inclusion criteria for human studies were established in the Population, Intervention, Comparator, Outcomes, and Study (PICOS) format, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [49]: (1) participants: children/adolescents/adults with a confirmed diagnosis of ASD as assessed by Diagnostic and Statistical Manual, Fourth Edition (DSM-IV), DSM-IV Text Revision (DSM-IV-TR), DSM, Fifth Edition (DSM-5) or International Classification of Diseases, Tenth Revision (ICD-10) criteria; (2) intervention: at least one of the following immunoregulatory/anti-inflammatory agents as single or add-on therapy: corticosteroids, neurosteroids, non-steroidal anti-inflammatory drugs (NSAID), minocycline, *N*-acetylcysteine (NAC), sulforaphane (SFN), omega-3 fatty acids; (3) comparator: placebo, or treatment as usual (TAU) *plus* placebo, corresponding to the intervention set-up; (4) outcome: validated rating scale; and (5) study design: randomized, placebo-controlled trials. Only studies in the English, German, Spanish,

or French languages were included. Exclusion criteria were (1) individuals with otherwise diagnosed ASD; (2) other immunoregulatory/anti-inflammatory agents; (3) agents requiring invasive techniques for their administration; (4) *in vivo* agents, such as probiotics; and (5) observational or quasi-experimental (non-randomized) studies.

## 2.3 Quality Assessment

The Jadad scale (also known as the Oxford quality scoring system) is considered as the standard method for evaluating randomized clinical trials [50] and consists of three items: (1) randomization; (2) blinding; and (3) description of patient's withdrawals/dropouts. Scores range from 0 to 5 points; a Jadad score of 0–2 indicates that the randomized clinical trial is of low quality, whereas a score of 3–5 indicates a high quality [50]. Two points relate to randomization and both are awarded if the answers to the following questions are 'yes': 'Was the study described as randomized?'; and 'Was the method of randomization appropriate?'. Two points relate to blinding and both are awarded if, again, the answers to the following questions are 'yes': 'Was the study described as double-blinded?'; and 'Was the blinding method appropriate?'. One point relates to a patient's withdrawals/dropouts.

## 3 Summary of Findings

In total, 18 studies were included in our review. The characteristics of the included studies are displayed in Table 1, and the total scores and quality ratings of the single studies are presented in Table 2.

### 3.1 Pharmacological Interventions

#### 3.1.1 Corticosteroids (Prednisolone)

Corticosteroids such as prednisolone are a class of steroid hormones secreted from the adrenal gland in response to stress. Since their discovery in the 1940s, corticosteroids have been used for the treatment of several immune and/or inflammatory diseases based on their immunosuppressive and/or anti-inflammatory effects [51]. To date, two randomized, placebo-controlled clinical trials have investigated the effectiveness of (add-on) prednisolone on improving core and associated symptoms in individuals with ASD [52, 53]. Overall, studies suggested a beneficial effect of prednisolone over placebo on *irritability*, *lethargy*, *stereotyped behavior*, and/or *hyperactivity* (as assessed by the change, from baseline to week 12 in the Aberrant Behavior Checklist-Community Edition [ABC-C] respective subscale scores) in at least, a subgroup of children with a regressive form of ASD (Table 1). In addition, a trend of prednisolone-specific improvement in the Language Development Assessment Tool (ADL) total score and in the Child Language Test in Phonology, Vocabulary, Fluency, and Pragmatics (ABFW) total of communicative acts subscale score was also found in, in particular, children with autistic disorder and a history of developmental regression (Table 1). Adverse effects were mild to moderate and included hypertension, hyperglycemia, and/or changes in appetite. Significant differences in relation to the frequency, type, or severity of adverse events were not found between individuals under (add-on) treatment with placebo and individuals under (add-on) treatment with prednisolone. The mechanism by which prednisolone exerts its anti-inflammatory and/or immunoregulatory action is not fully understood. Increasing evidence from animal and human studies has suggested that corticosteroids may modulate microglial activation, and also restore Treg/Th17 cell imbalances [54–56]. Corticosteroids are also capable of increasing the number of mature NK cells [57], and of reducing blood levels of pro-inflammatory cytokines, such as IL-6 and/or IFN $\gamma$ , within their first weeks of administration.

#### 3.1.2 Neurosteroids (Pregnenolone)

Pregnenolone is a steroid hormone precursor that is synthesized in different steroidogenic tissues, the brain, and in lymphocytes. It is known to act as an anti-inflammatory and/or immunoregulatory agent in several (neuro)inflammatory diseases [58]. To date, only one randomized, placebo-controlled study has investigated the effectiveness of pregnenolone for the management of core and related symptoms in subjects with ASD [59]. In this study, a total of 59 medication-naïve children and adolescents with ASD and moderate-to-high levels of irritability were randomly allocated

to either risperidone + placebo ( $n = 29$ ), or to risperidone + pregnenolone ( $n = 30$ ), and followed up over a 10-week period (Table 1). The primary outcome measure included the change, from baseline to week 10, in the ABC irritability subscale score; secondary outcome measures included the change in other ABC subscale scores (i.e., hyperactivity, lethargy, stereotypy, inappropriate speech). After 10 weeks of continuous treatment, individuals allocated to risperidone + pregnenolone showed a statistically significantly higher improvement in *irritability*, *stereotyped behavior*, and *hyperactivity* compared to those allocated to risperidone + placebo. A placebo- or pregnenolone-specific effect on lethargy and/or inappropriate speech was however, not found (Table 1). Pregnenolone had a good safety profile and was well tolerated; adverse events were mild to moderate, and included changes in appetite, dizziness, rash, diarrhea, headache, or abdominal pain. Again, both study groups did not statistically differ in relation to the frequency, severity, or type of adverse effects. The mechanism by which pregnenolone exerts its anti-inflammatory and/or immunoregulatory action is again, not fully understood. Interestingly, several reports have suggested that neurosteroids, such as pregnenolone, are also able to suppress microglial and Th17 cells proinflammatory activation in humans, and in murine models of autoimmune conditions [60].

#### 3.1.3 Non-steroidal Anti-inflammatory Drugs [Celecoxib]

Celecoxib acts as a NSAID that selectively inhibits the cyclooxygenase (COX)-2 enzyme. This agent is better tolerated than steroidal anti-inflammatory drugs, and is associated with a lower risk of gastrointestinal bleeding [61]. To date, only one randomized, double-blind, placebo-controlled trial has investigated the effectiveness of celecoxib on ASD symptomatology [62]. In this study, a total of 40 medication-naïve children with autistic disorder and moderate-to-high levels of irritability were randomly allocated to either risperidone + placebo ( $n = 20$ ), or to risperidone + celecoxib ( $n = 20$ ), and followed up over a 10-week period (Table 1). The primary outcome measure was the change, from baseline to week 10, in the ABC irritability subscale score, while secondary outcome measures included the change in other ABC subscale scores (i.e., lethargy/social withdrawal, stereotypic behavior, hyperactivity, inappropriate speech). After 10 weeks of continuous treatment, individuals allocated to risperidone + celecoxib showed a statistically significantly higher improvement in *irritability*, *lethargy/social withdrawal*, and *stereotyped behavior* compared to individuals allocated to risperidone + placebo. However, a celecoxib-specific improvement in other symptoms, such as hyperactivity and/or inappropriate speech was not found (Table 1). Celecoxib was well tolerated and both study groups did not statistically differ in relation to the frequency, severity, or

**Table 1** Summary of randomized, placebo-controlled studies on immunomodulatory/anti-inflammatory therapies in autism spectrum disorder

| Agent                          | Diagnosis (diagnostic criteria applied)  | Medication-free                                  | N   | Age range              | Intervention period (weeks) | Intervention (dose)  | Outcome measures    | Outcomes  |
|--------------------------------|--|--|---|------------------------|-----------------------------|--|---------------------|---|
| <i>Prednisolone</i>            |  |  |   |                        |                             |  |                     |   |
| Malek et al. (2020) [52]       | ASD (regressive) [DSM-5] ADI-R   | Only antipsychotic-native                        | Placebo (n = 13)<br>Prednisolone (n = 13) | Age range: 3–12 years  | 12                          | Risperidone: 0.5–2 mg/day<br>Prednisolone: 1 mg/kg/day   | ABC<br>CARS         | Prednisolone-specific improvement in:<br>ABC: irritability (p = 0.026), stereotypies (p = 0.026), hyperactivity (p = 0.039), lethargy (p < 0.001)<br>CARS total score (p < 0.001) |
| Rocha-Brito et al. (2021) [53] | Autistic disorder (DSM-IV) NG  | No<br>*Pretreated with oral albendazole (5 days) | Placebo (n = 20)<br>Prednisolone (n = 18) | Age range: 3–7 years   | 24                          | Prednisolone: 1 mg/kg 8 weeks, 9–16 weeks 1 mg/kg/day every other day, final 8 weeks: gradual dose reduction on alternate days | ADL<br>ABFW<br>CARS | Trend of prednisolone-specific improvement in:<br>ADL (p = 0.057)<br>ABFW total of communicative acts (p = 0.054)   |
| <i>Pregnenolone</i>            |  |  |   |                        |                             |  |                     |   |
| Ayatollahi et al. (2020) [59]  | ASD (DSM-5) ADI-R<br>Moderate-to-high levels of irritability                   | Yes  | Placebo (n = 29)<br>Pregnenolone (n = 30) | Age range: 11–17 years | 10                          | Risperidone: 0.5–3.5 mg/day<br>Pregnenolone: 200 mg/day  | ABC                 | Pregnenolone-specific improvement in:<br>ABC: irritability (p = 0.025), stereotypies (p = 0.029), hyperactivity (p = 0.004)   |
| <i>Celecoxib</i>               |  |  |   |                        |                             |  |                     |   |
| Asadabadi et al. (2013) [62]   | Autistic disorder (DSM-IV) ADI-R<br>Moderate-to-high levels of irritability    | Yes  | Placebo (n = 20)<br>Celecoxib (n = 20)    | Age range: 4–12 years  | 10                          | Risperidone: 0.5–2 mg/day<br>Celecoxib: 300 mg/day   | ABC                 | Celecoxib-specific improvement in:<br>ABC: irritability (p < 0.001), lethargy/social withdrawal (p < 0.001), stereotypic behavior (p < 0.001)                                     |
| <i>Minocycline</i>             |  |  |   |                        |                             |  |                     |   |
| Ghaleiha et al. (2016) [67]    | Autistic disorder (DSM-IV-TR) ADI-R<br>Moderate-to-high levels of irritability | Yes  | Placebo (n = 23)<br>Minocycline (n = 23)  | Age range: 4–12 years  | 10                          | Risperidone: 0.5–2 mg/day<br>Minocycline: 50 mg/day  | ABC                 | Minocycline-specific improvement in:<br>ABC: irritability (p = 0.003), hyperactivity/non-compliance (p = 0.002)   |
| <i>N-Acetylcysteine</i>        |  |  |   |                        |                             |  |                     |   |



Table 1 (continued)

| Agent                                     | Diagnosis (diagnostic criteria applied)<br>Structured/semi-structured diagnostic interview        | Medication-free | N   | Age range | Intervention period (weeks)                      | Intervention (dose)                                     | Outcome measures   | Outcomes |
|---|---|-----------------|---|-----------|--|---|--|----------|
| Hardan et al. (2012) [75]                 | Autistic disorder (DSM-IV-TR)<br>ADI-R, ADOS<br>CGI-S $\geq 4$                                    | Yes             | Placebo ( $n = 15$ )<br>NAC ( $n = 14$ )<br>Age range: 3–11 years | 12        | NAC: 900–2700 mg/day                             | ABC<br>CGI-I<br>CGI-S<br>SRS<br>RBS-R                   | NAC-specific improvement in:<br>ABC: irritability ( $p < 0.001$ ), stereotyped/repetitive behavior ( $p = 0.096$ )<br>RBS: stereotypies ( $p = 0.014$ )<br>SRS: social cognition ( $p = 0.037$ ), mannerisms ( $p = 0.045$ )<br>NAC-specific improvement in:<br>ABC: irritability ( $p = 0.035$ )<br>NAC-specific improvement in:<br>ABC: irritability ( $p = 0.01$ ), hyperactivity ( $p = 0.01$ )<br>No placebo- or NAC-specific improvement in any of the primary or secondary outcome measures |          |
| Ghanizadeh and Moghimi-Sarani (2013) [76] | Autistic disorder (DSM-IV-TR)<br>ADI-R  | Yes             | Placebo ( $n = 14$ )<br>NAC ( $n = 17$ )<br>Age range: 3–16 years | 8         | NAC: 1200 mg/day                                 | ABC   | NAC-specific improvement in:<br>ABC: irritability ( $p = 0.035$ )<br>NAC-specific improvement in:<br>ABC: irritability ( $p = 0.01$ ), hyperactivity ( $p = 0.01$ )<br>No placebo- or NAC-specific improvement in any of the primary or secondary outcome measures   |          |
| Nikoo et al. (2015) [77]                  | Autistic disorder (DSM-IV-TR)<br>ADI-R<br>ABC-I $\geq 12$   | Yes             | Placebo ( $n = 20$ )<br>NAC ( $n = 20$ )<br>Age range: 4–12 years | 10        | Risperidone: 0.5–2 mg/day<br>NAC: 600–900 mg/day | ABC   | NAC-specific improvement in:<br>ABC: irritability ( $p = 0.01$ ), hyperactivity ( $p = 0.01$ )<br>No placebo- or NAC-specific improvement in any of the primary or secondary outcome measures  |          |
| Wink et al. (2016) [78]                   | ASD (autistic disorder, Asperger's disorder, PDD-NOS) (DSM-IV)<br>ADI-R<br>CGI-S (moderately ill) | No              | Placebo ( $n = 12$ )<br>NAC ( $n = 16$ )<br>Age range: 3–12 years | 12        | NAC: 60 mg/kg/day                                | ABC<br>SRS<br>VABS-II<br>CGI-I                          | No placebo- or NAC-specific improvement in any of the primary or secondary outcome measures  |          |
| Dean et al. (2017) [79]                   | Autistic disorder (DSM-IV-TR)   | No              | Placebo ( $n = 35$ )<br>NAC ( $n = 36$ )<br>Age range: 3–9 years  | 24        | NAC: 500 mg/day                                  | SRS<br>CCC<br>RBS-R<br>DBC-P<br>PGI-I<br>CGI-I<br>CGI-S | No placebo- or NAC-specific improvement in any of the primary or secondary outcome measures  |          |

Sulfuraphane

Table 1 (continued)

| Agent   | Diagnosis (diagnostic criteria applied)<br>Structured/semi-structured diagnostic interview | Medication-free | N<br>Age range  | Intervention period (weeks) | Intervention (dose)   | Outcome measures   | Outcomes   |
|---|--|-----------------|---|-----------------------------|---|--|--|
| Singh et al. (2014) [84]                              | Autistic disorder (DSM-IV)<br>ADOS<br>Moderate-to-severe                                   | No              | Placebo (n = 14)<br>SFN (n = 26)<br>Age range: 13–27 years    | 18                          | SFN: 50–150 µM/day  | ABC<br>SRS<br>CGI-I  | SFN-specific improvement in:<br>ABC: total score (p < 0.001), irritability, lethargy, stereotypies, hyperactivity<br>SRS: total score (p = 0.017), awareness, communication, motivation, mannerisms<br>CGI-I: social interaction (p = 0.007), aberrant/abnormal behavior (p = 0.014), verbal communication (p = 0.015)<br>SFN-specific improvement in:<br>ABC: irritability (p < 0.001), hyperactivity (p = 0.015)<br>SFN-specific improvement in:<br>ABC: total score (p = 0.02), hyperactivity (p = 0.04), inappropriate speech (p = 0.01) |
| Montazmanesh et al. (2020) [85]                       | ASD (DSM-V)<br>ADI-R   | Yes             | Placebo (n = 30)<br>SFN (n = 30)<br>Age range: 4–12 years     | 10                          | Risperidone: 0.25–3.5 mg/day<br>SFN: 50–100 µM/day  | ABC  | SFN-specific improvement in:<br>ABC: irritability (p < 0.001), hyperactivity (p = 0.015)   |
| Zimmerman et al. (2021) [86]                          | ASD (DSM-V)<br>ADOS-2<br>VABS-II<br>Moderate-to-severe                                     | No              | Placebo (n = 23)<br>SFN (n = 22)<br>Age range: 3–12 years     | 15                          | SFN: tablets containing active myrosinase + broccoli seed and sprout extracts (≈ 15 µmol/L SFN) | OASIS<br>ABC<br>SRS-2  | SFN-specific improvement in:<br>ABC: total score (p = 0.02), hyperactivity (p = 0.04), inappropriate speech (p = 0.01)   |
| <i>Omega-3 fatty acids</i><br>Bent et al. (2011) [92] | ASD (DSM-IV-TR)<br>ADOS<br>SCQ<br>Moderate-severe  | No              | Placebo (n = 12)<br>Omega-3 (n = 13)<br>Age range: 5–17 years | 12                          | Omega-3: 350 mg EPA + 230 mg DHA<br>Placebo: safflower oil<br>Twice daily                       | ABC<br>BASC<br>CGI-I<br>SRS<br>Peabody Picture Vocabulary test<br>Expressive Vocabulary test | No placebo- or intervention-specific effect in any of the primary or secondary outcome measures  |

Table 1 (continued)

| Agent                       | Diagnosis (diagnostic criteria applied)<br>Structured/semi-structured diagnostic interview | Medication-free | N  | Age range | Intervention period (weeks)   | Intervention (dose)                          | Outcome measures   | Outcomes |
|-----------------------------|--|-----------------|--|-----------|---|--|--|----------|
| Bent et al. (2014) [93]     | ASD (NG)<br>ADOS, ADI-R<br>SCQ > 12<br>ABC-H ≥ 20  | No              | Placebo (n = 28)<br>Omega-3 (n = 29)<br>Age range: 5–8 years   | 6         | Omega-3: 350 mg EPA + 230 mg DHA<br>Placebo: safflower oil<br>Twice daily             | ABC<br>SRS<br>CGI-I                          | Omega-3-specific improvement in:<br>ABC (parent rated): stereotypies (p = 0.05), lethargy (p = 0.01)<br>Omega-3-specific worsening in externalizing behaviors (p = 0.02) |          |
| Mankad et al. (2015) [94]   | ASD (DSM-IV-TR)<br>ADOS<br>ADI-R   | No              | Placebo (n = 19)<br>Omega-3 (n = 18)<br>Age range: 2–5 years   | 24        | Omega-3: 0.75–1.5 g EPA+DHA/day<br>Placebo: refined olive oil and medium chain TG/day | PDDBI<br>BASC-2<br>VABS-II<br>PLS-4<br>CGI-I | Omega-3-specific improvement in:<br>ABC<br>SRS-2<br>SPM<br>BPFAS   |          |
| Mazahery et al. (2019) [95] | ASD (DSM-5)  | No              | Placebo (n = 16)<br>Omega-3 (n = 23)<br>Age range: 2.5–8 years | 12 months | Omega-3: 722 mg DHA/day   | ABC<br>SRS-2<br>SPM<br>BPFAS                 | Omega-3-specific improvement in:<br>ABC: irritability (p = 0.001), lethargy (p = 0.02)   |          |
| Doaei et al. (2021) [96]    | Autistic disorder (DSM-IV-TR)<br>ADOS<br>Moderate-to-severe                                | Yes             | Placebo (n = 26)<br>Omega-3 (n = 28)<br>Age range: 5–15 years  | 8         | Omega-3: 540 mg EPA + 360 mg DHA/day<br>Placebo: medium chain TG/day                  | GARS-2                                       | Omega-3-specific improvement in:<br>GARS-2: total score behaviors (p = 0.02), social communication (p = 0.02)  |          |

ABC Aberrant Behavior Checklist, ABC-H ABC-Hyperactivity subscale, ABC-I ABC-Irritability subscale, ABFW Child Language Test in Phonology, Vocabulary, Fluency, and Pragmatics, ADI-R Autism Diagnostic Interview-Revised, ADL Language Development Assessment, ADOS Autism Diagnostic Observation Schedule, ASD autism spectrum disorder, BASC Behavior Assessment System for Children, BPFAS Behavioral Pediatrics Feeding Assessment Scale, CARS Childhood Autism Rating Scale, CCC Children's Communication Checklist, CGI-I Clinical Global Impression-Improvement, CGI-S Clinical Global Impression-Severity, DBC-P Development Behavior Checklist, DHA docosahexaenoic acid, DSM Diagnostic and Statistical Manual of Mental Disorders, DSM-IV DSM Fourth Edition, DSM-IV-TR DSM Fourth Edition Text Revision, EPA eicosapentaenoic acid, GARS Gilliam Autism Rating Scale, NAC N-acetylcysteine, NG not given, OASIS Overall Anxiety Severity and Impairment Scale, PDDBI Pervasive Developmental Disorder Behavior Inventory, PDD-NOS Pervasive Developmental Disorder Not Otherwise Specified, PGI-I Patient Global Impression of Improvement Scale, PLS-4 Preschool Language Scale, RBS-R Repetitive Behavior Scale-Revised, SCQ Social Communication Questionnaire, SFV sulforaphane, SPM Sensory Processing Measure questionnaire, SRS Social Responsiveness Scale, TG triglycerides, VABS-II Vineland Adaptive Behavior Scale, Second Edition. \*In the study performed by Rocha-Brito and colleagues (2021) [53], patients were treated, before the starting of the trial, with oral albenzazole for five days.



**Table 2** Quality assessment of included studies

|   | Study agent              | Quality score | Interpretation |
|---|--------------------------|---------------|----------------|
| Malek et al. (2020) [52]                  | Prednisolone             | ****          | High quality   |
| Rocha-Brito et al. (2021) [53]            |                          | *****         | High quality   |
| Ayatollahi et al. (2020) [59]             | Pregnenolone             | *****         | High quality   |
| Asadabadi et al. (2013) [62]              | Celecoxib                | *****         | High quality   |
| Ghaleiha et al. (2016) [67]               | Minocycline              | *****         | High quality   |
| Hardan et al. (2012) [75]                 | <i>N</i> -Acetylcysteine | *****         | High quality   |
| Ghanizadeh and Moghimi-Sarani (2013) [76] |                          | *****         | High quality   |
| Nikoo et al. (2015) [77]                  |                          | *****         | High quality   |
| Wink et al. (2016) [78]                   |                          | *****         | High quality   |
| Dean et al. (2017) [79]                   |                          | *****         | High quality   |
| Singh et al. (2014) [84]                  | Sulforaphane             | ****          | High quality   |
| Momtazmanesh et al., (2020) [85]          |                          | *****         | High quality   |
| Zimmerman et al. (2021) [86]              |                          | ****          | High quality   |
| Bent et al. (2011) [92]                   | Omega-3                  | *****         | High quality   |
| Bent et al. (2014) [93]                   |                          | *****         | High quality   |
| Mankad et al. (2015) [94]                 |                          | *****         | High quality   |
| Mazahery et al. (2019) [95]               |                          | *****         | High quality   |
| Doaei et al. (2021) [96]                  |                          | *****         | High quality   |

type of adverse effects, which included changes in appetite, abdominal pain, dizziness, insomnia, nausea, and/or sedation. A possible mechanism, by which celecoxib improves these symptoms in individuals with autistic disorder is, again, the inhibition of microglial/monocyte [63] and/or Th17 cells activation [64]. Interestingly, several reports have suggested that different proinflammatory cytokines, such as IL-1 $\beta$ , IL-6, and/or IL-17A, could serve as indicators for predicting clinical response to celecoxib in individuals with immune-mediated conditions, such as ankylosing spondylitis [65].

### 3.1.4 Minocycline

Minocycline is a second-generation tetracycline antibiotic with well-known antioxidant, immunoregulatory, and/or anti-inflammatory properties [66]. To date, only one randomized, double-blind, placebo-controlled trial has assessed the effectiveness of minocycline on improving core and associated symptoms in individuals with ASD [67]. In this study, a total of 46 medication-naïve children diagnosed with autistic disorder and with moderate-to-high levels of irritability were randomly allocated to either risperidone + placebo ( $n = 23$ ), or to risperidone + minocycline ( $n = 23$ ), and followed up over a 10-week period (Table 1). The primary outcome measure was the change, from baseline to week 10, in the ABC irritability subscale score, while secondary outcome measures included the change in other ABC subscale scores (i.e., lethargy/social withdrawal, stereotypic behavior, hyperactivity, inappropriate speech). At the end

of the intervention phase at week 10, a significantly higher improvement in *irritability* and *hyperactivity* was found in the subgroup of patients allocated to (add-on) minocycline when compared to those allocated to (add-on) placebo. An intervention-specific effect on the other symptoms assessed was however, not found (Table 1). Minocycline had a good safety profile and was well tolerated, adverse events were mild to moderate and included diarrhea, headache, increase in appetite, dizziness, insomnia, nausea, and/or sedation. Both study groups did not statistically differ in relation to the frequency, severity, or type of adverse effects. Again, studies have found that minocycline is able to inhibit microglial/monocyte proinflammatory activation [68–70] and to regulate the Th17/Treg cells axis, decreasing the levels of several proinflammatory cytokines such as IL-1 $\beta$ , IL-6, TNF $\alpha$ , IFN $\gamma$ , and/or IL-17A, both in the brain and in the periphery [71].

### 3.1.5 *N*-Acetylcysteine

NAC is a synthetic *N*-acetyl derivative of the endogenous amino acid *L*-cysteine [72], which acts as a precursor of the antioxidant enzyme glutathione (i.e., the most abundant antioxidant in the brain) [73]. An increasing body of evidence suggests that NAC may also act as an immunoregulatory and/or anti-inflammatory agent [74]. Therefore, several randomized, placebo-controlled clinical trials have investigated the effectiveness of (add-on) treatment with NAC on improving core and associated symptoms in individuals with ASD (Table 1). In general, studies suggest that (add-on)

treatment with NAC may be beneficial for the management of *irritability* [75–77] in, at least, a subgroup of children and adolescents with autistic disorder (Table 1). In all these studies, children and adolescents were medication-naïve, and the diagnosis of autistic disorder was previously confirmed by a (semi)-structured interview (i.e., the Autism Diagnostic Interview-Revised [ADI-R], and/or the Autism Diagnostic Observation Schedule [ADOS]). The improvement in irritability was assessed by the change, from baseline until week 8 [76], 10 [77], or 12 [75] in the respective ABC subscale score. NAC was administered at a dose range of 600–2700 mg/day (Table 1). In only one of four studies assessing irritability as an outcome measure, (add-on) treatment with NAC was not associated with a significantly higher improvement in this symptom, compared with placebo (Table 1) [78]. In this study, children were not medication-naïve, and were diagnosed with ASD (i.e., autistic disorder, Asperger's disorder, and/or pervasive developmental disorder not otherwise specified [PDD-NOS]) (Table 1), something which could have influenced the findings. Mixed findings were found for other symptoms assessed, such as hyperactivity (i.e., ABC subscale score), stereotyped/repetitive behavior (i.e., ABC and/or Repetitive Behavior Scale [RBS] subscale scores), mannerisms (i.e., Social Responsiveness Scale [SRS] subscale score), and/or social cognition (SRS subscale score) (Table 1) [75–79]. Differences in the duration of the treatment period, in the questionnaires/scales used for assessing symptoms, and/or in the dosage of the study agent could have also influenced results. In all studies, NAC had a good safety profile and was well tolerated; significant differences in relation to the frequency and/or severity of adverse effects were not found between individuals treated with (add-on) placebo, and those treated with (add-on) NAC. Adverse effects were mild to moderate and included gastrointestinal symptoms (e.g., abdominal pain, diarrhea and/or constipation, changes in appetite, nausea), headache, rash, insomnia, and/or fatigue. The mechanism of action by which NAC improves irritability in individuals with autistic disorder is not fully understood. NAC can reverse microglial proinflammatory activation [80], and several studies have suggested that NAC could also regulate the Treg/Th17 axis in individuals with inflammatory conditions, such as chronic obstructive pulmonary disease (COPD) [81]. In addition, animal models of experimental autoimmune encephalomyelitis have also demonstrated a suppressive action of NAC on Th17 cells [82].

## 3.2 Dietary Interventions

### 3.2.1 Sulforaphane

SFN is an isothiocyanate derived from *Brassica* vegetables (in particular, from broccoli) with antioxidant,

immunoregulatory, and/or anti-inflammatory properties [83]. To date, three randomized, placebo-controlled trials have assessed the effectiveness of this agent on improving core and/or related symptoms in individuals with ASD (Table 1). Overall, studies suggest that (add-on) treatment with SFN may improve *hyperactivity* in children, adolescents, and adults with autistic disorder and/or ASD [84, 85]. In all these studies, the improvement in hyperactivity was assessed by the change from baseline until week 10 [84], 1586, or 18 [85] in the respective ABC subscale score (Table 1). The dose of SFN ranged between 50 and 150  $\mu\text{M}$ /day; one study used glucoraphanin-rich broccoli seed extract tablets containing myrosinase, instead of SFN [86] (Table 1). Mixed findings were found for other symptoms assessed, such as irritability, lethargy, stereotyped/repetitive behavior (i.e., ABC subscale scores), mannerisms, awareness, motivation, social communication (i.e., SRS subscale scores), and social interaction, aberrant/abnormal behavior, and/or verbal communication (CGI-I subscale scores) (Table 1). Differences in the duration of the treatment period, in the questionnaires/scales used for assessing symptoms, and in the dosage and/or composition of the study agent could have influenced the findings. SFN had a good safety profile, and was well tolerated; adverse effects included abdominal pain, increased flatulence, constipation, diarrhea, vomiting, increased appetite, weight gain, headache, irritability, increased aggression, dizziness, sedation, insomnia, rashes, exacerbation of seasonal allergies, and/or fever. Significant differences in relation to the frequency and/or severity of adverse effects were not found between individuals allocated to (add-on) placebo, and those allocated to (add-on) SFN. The mechanism of action by which SFN improves these symptoms in individuals with ASD is not known at all. SFN may exert an anti-inflammatory effect on microglia [87]. Interestingly, in a study performed on Black and Tan Brachyury (BTBR) T+ Itpr3tf/J mice (i.e., a strain of mouse model that is most noted for its phenotypic similarities to humans on the ASD scale), SFN was able to ameliorate autism-like behaviors (i.e., reduced self-grooming/marble burying behavior, increased social interaction) through suppression of Th17-related signaling both in the periphery, and in the brain (i.e., SFN-treated BTBR mice were characterized by a reduced expression of *STAT3*, *RORC*, *IL-17A*, and/or *IL-23R* in  $\text{CD4}^+$  Th cells) [88]. In another study performed on children with ASD, SFN was associated with nuclear factor erythroid 2-related factor 2 (Nrf2) stimulation, resulting in an inhibitory effect on nitrate stress markers and pro-inflammatory cytokines [89].

### 3.2.2 Omega-3 Fatty Acids

Omega-3 polyunsaturated fatty acids (PUFAs) include  $\alpha$ -linolenic acid (ALA), stearidonic acid (SDA),

eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Due to their well-known anti-inflammatory actions [90, 91], most trials assessing dietary interventions in ASD have used omega-3 PUFAs as the study agent, but with mixed findings (Table 1). (Add-on) treatment with omega-3 fatty acids resulted beneficial for the management of *lethargy* in two of three studies that used the change in the ABC respective subscale score as the outcome measure [93, 95]. In both studies, study participants were children (age range 2.5–8 years) diagnosed with an ASD, and omega-3 supplements consisted of DHA (with or without EPA). The daily dose of DHA was  $\cong$  460–722 mg/day, and the daily dose of EPA was  $\cong$  700 mg/day (Table 1). In the remaining study [92], study participants were children and adolescents diagnosed with a moderate-to-severe ASD, and the sample size was lower, something which could have influenced the findings (Table 1). (Add-on) treatment with omega-3 was also beneficial for the management of *stereotyped behaviors* in two of four studies assessing this symptom (by the change in the ABC and/or in the GARS-2 respective subscale scores) (Table 1) [93, 96]. Study participants were children and adolescents (age range 5–15 years) diagnosed with ASD or with autistic disorder. In both studies, the intervention product was composed of DHA (dose range 360–460 mg/day) + EPA (dose range 540–700 mg/day) (Table 1). Mixed findings were found for other symptoms assessed, such as irritability (i.e., ABC subscale score), and/or social communication (i.e., GARS-2 subscale score) (Table 1). Differences in the duration of the treatment period, in the scales used for assessing symptoms, and/or in the dosage or composition of the study agent could have influenced results. Interestingly, in a study performed on children with ASD [94], (add-on) treatment with 750 mg EPA + 1500 mg DHA/day was associated with a worsening in externalizing behaviors (BASC-2) at week 24, when compared with placebo. In this study, all participants were younger than 5 years of age, and omega-3 was administered at higher doses in comparison with the other studies, making possible that omega-3 fatty acids worsens externalizing behaviors in this age group, when given at high doses. Moreover, the majority of study participants were minimally verbal and therefore, potential gastrointestinal distress may have been captured as reports of externalizing behaviors. Omega-3 fatty acids had a good safety profile and were well tolerated, no serious adverse events were reported during the study. Most adverse events reported were mild to moderate and included neuropsychiatric symptoms (e.g., decreased energy, headache), sleep disturbances (e.g., insomnia, early awakening), nutritional or gastrointestinal symptoms (e.g., changes in appetite, abdominal pain), dermatological, or others, such as eye swelling. No statistically significant differences in relation to the frequency and characteristics of adverse events were found between patients allocated to

(add-on) placebo, and those allocated to (add-on) omega-3 fatty acids. The mechanism of action by which omega-3 fatty acids exert their action is not fully understood. Again, evidence suggests that omega-3 fatty acids exert their action by (at least in part) increasing the expression of FoxP3 and the differentiation of Tregs, while inhibiting Th17 promotion, and reducing IL-17A production [97]. In addition, these compounds have also been found to reverse microglial pro-inflammatory activation [98].

## 4 Discussion

Overall, our findings suggest a beneficial effect of (add-on) treatment with prednisolone, pregnenolone, celecoxib, minocycline, and/or NAC on *irritability*; a beneficial effect of (add-on) treatment with prednisolone, pregnenolone, minocycline, and/or SFN on *hyperactivity*; a beneficial effect of (add-on) treatment with prednisolone, celecoxib, and/or omega-3 fatty acids on *lethargy*, and a beneficial effect of (add-on) treatment with prednisolone, pregnenolone, celecoxib, and/or omega-3 fatty acids on *stereotyped behavior*, over placebo. All agents had a good safety profile and were well tolerated; significant differences in relation to the frequency and/or severity of adverse effects were not found between individuals treated with placebo and those treated with the different above-mentioned compounds. The mechanism, by which all these agents exert their action is not fully understood. Interestingly, all agents are able to inhibit microglial proinflammatory activation, and to restore Treg/Th17 imbalances in at least a subgroup of individuals with ASD, decreasing the levels of proinflammatory cytokines such as IL-6 and/or IL-17A in both the brain, and the periphery. Although encouraging, findings should be considered in light of several limitations. The majority of studies included children and adolescents as participants and therefore, findings may not be applicable to adults with ASD. Moreover, the sample size was low in the majority of studies, and most studies excluded non-verbal participants with severe intellectual disabilities, and included study participants with different diagnoses (i.e., ASD vs. autistic disorder vs. PDD-NOS vs. Asperger's syndrome). Therefore, findings may only be applicable to a small subgroup of individuals living with this condition. The severity of baseline ASD symptoms was inconsistent among trials, and the use of concomitant psychiatric medications and/or of behavioral treatments was allowed in several studies, something which could have influenced the findings. Differences in the active treatment composition and/or in the dosage used, as well as in the duration of the treatment period, could also have influenced the results. Unfortunately, baseline levels of biological (immune/inflammatory) markers were not assessed in the majority of studies. This would have been interesting in

order to identify (immune/inflammatory) predictors of treatment response and thus, to also identify those individuals who would benefit from a particular treatment regimen, at baseline (personalized medicine).

## 5 Conclusions and Future Perspectives

The evidence that immune dysfunction may play a role in the pathophysiology of at least a subgroup of individuals with ASD is now considerable. An increasing body of evidence has supported the existence of a chronic activation of not only microglia, but also of the monocyte/macrophage system in individuals with ASD, across the lifespan. In addition, abnormal levels of several lymphocyte subpopulations (e.g., Tregs, Th17 cells) have been repeatedly demonstrated. Therefore, immunoregulatory and/or anti-inflammatory agents may represent a possibility for more personalized treatment regimens among individuals diagnosed with this condition. Several randomized, placebo-controlled trials on the effectiveness of (add-on) treatment with prednisolone, pregnenolone, celecoxib, minocycline, NAC, SFN, and/or omega-3 fatty acids on core and related symptoms of ASD have been performed, with promising findings. However, larger randomized, placebo-controlled trials including, for example, more homogeneous study populations, and longer periods of follow-up are urgently needed to confirm the findings.

### Declarations

**Funding** No funding was secured for this study. Open access publication was supported by the Group of Psychiatry, Mental Health and Addiction of Vall d'Hebron Research Institute, Barcelona, Catalonia, Spain.

**Conflict of interest** All authors have no financial relationships to disclose relevant to this article. Josep Antoni Ramos-Quiroga was on the speakers' bureau and/or acted as a consultant for Janssen-Cilag, Novartis, Shire, Takeda, Bial, Shionogi, Sincrolab, Novartis, BMS, Medice, Rubió, Uriach, Technofarma and Raffo in the last 3 years, and also received travel awards (air tickets + hotel) from Janssen-Cilag, Rubió, Shire, Takeda, Shionogi, Bial and Medice for taking part in psychiatric meetings. The Department of Psychiatry chaired by Josep Antoni-Ramos-Quiroga received unrestricted educational and research support from Janssen-Cilag, Shire, Oryzon, Roche, Psious, and Rubió in the last 3 years.

**Availability of data and material** Data sharing was not applicable to this article as no datasets were generated and/or analyzed during the current study.

**Consent to participate** Not applicable.

**Ethics approval** Not applicable.

**Consent for publication** Not applicable.

**Code availability** Not applicable.

**Author contributions** GAH: conceptualization, data curation, investigation, methodology, validation, visualization, writing—original draft, review, and editing. LG: data curation, investigation, methodology, validation, visualization: review, and editing. JARQ: writing—review, and editing. All authors read and approved the final submitted manuscript and agree to be accountable for this work.

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