



Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders

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Summary Multiple studies now demonstrate that autism is medically characterized, in part, by immune system dysregulation, including evidence of neuroglial activation and gastrointestinal inflammation. This neuroglial process has further been characterized as neuroinflammation. In addition, a subset of autistic children exhibit higher than average levels of androgens. Spironolactone is an aldosterone antagonist and potassium-sparing diuretic with a desirable safety profile. It possesses potent anti-inflammatory and immune modifying properties that might make it an excellent medical intervention for autism spectrum disorders. Furthermore, spironolactone demonstrates substantial anti-androgen properties that might further enhance its appeal in autism, particularly in a definable subset of hyperandrogenic autistic children. One case report is briefly reviewed demonstrating objective clinical improvements in an autistic child after spironolactone administration. Additional research in controlled trials is now needed to further define the risks and benefits of spironolactone use in children with autism.

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Abbreviations: DHEA, dehydroepiandrosterone; BDNF, brain derived neurotrophic factor; IL-10, Interleukin-10; TNF- α , tumor necrosis factor alpha; MCP-1, macrophage chemoattractant protein-1; CSF, cerebrospinal fluid; IVIG, intravenous immunoglobulin; ALPS, autoimmune lymphoproliferative syndrome; DHT, dihydrotestosterone; ABC, aberrant behavior checklist.

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Background

Classical thinking regarding autism defines it as a developmental disorder with abnormalities in language, socialization, and stereotypical and unusual behaviors [1,2]. Two recent studies demonstrate that the prevalence of autism is increasing, with

indications that as many as 1–2% of boys might be affected in the United States and England [3,4]. Both studies confirm observations that approximately four times as many males are affected as females. In addition, many children with autism have evidence of immune dysregulation, neuroinflammation and gastrointestinal inflammation. Furthermore, several studies confirm that at least a subset of autistic children is documented to have either elevated testosterone and/or dehydroepiandrosterone (DHEA) levels [5–7].

Hypothesis

Autism is characterized by both increased inflammation and immune dysregulation. In addition, a subset of autistic children exhibit higher than average androgen levels. Spironolactone possesses potent anti-inflammatory, immunologic, and hormone modifying properties that might make it a desirable medical intervention for autism spectrum disorders.

Evidence and discussion

Immune dysregulation in autism

Recognition has been growing over the past 30 years that autism spectrum disorders are often associated with abnormal immune function. In 1976, Stubbs first noted the absence of a normal response to rubella vaccination in a group of children with autism [8]. Then in 1977, Stubbs and Crawford went on to demonstrate suppressed responsiveness to *in vitro* phytohemagglutinin stimulation of lymphocyte cultures [9]. In as early as 1982, Weizman and colleagues demonstrated abnormal cell-mediated immune response to brain tissue in autistic individuals [10]. The understanding of cell-mediated immune dysregulation in autism was expanded by 1986 to include reduced responsiveness in lymphocyte blastogenesis, decreased numbers of T-lymphocytes, and an altered ratio of helper to suppressor T-cells [11]. Warren next demonstrated reduced natural killer cell activity in autistic children [12]. As further evidence regarding immune abnormalities mounted in the neuroimmunopsychiatry literature [13,14], Francesetti and colleagues advanced the hypothesis that the immune system might play a role in the pathogenesis of both schizophrenia and autism [15].

By the 1990s, the science of immunology was rapidly expanding, spurred on by the successes of AIDS-related research and large governmental

grants. This permitted a more advanced investigation of the neuroimmunological aspects of autism. In 1991, Singh et al. first reported abnormal cytokine levels, which indirectly indicated activation of a subpopulation of T-cells in autism [16]. The autoimmune aspect of autism was furthered by Warren et al. when they discovered abnormalities in T-cells compatible with an autoimmune disorder [17]. Scifo et al. reported a favorable decrease in autism symptoms directly related to the normalization of immune abnormalities following intervention with naltrexone [18]. This treatment resulted in a significant increase in the number of T-helper-inducer cells ($CD4^+CD8^-$) and a substantial reduction in the number of T-cytotoxic-suppressor cells ($CD4^-CD8^+$), thereby normalizing the CD4/CD8 ratio. Singh reported elevated antibodies to neuron-axon filament protein (anti-NAFP) and glial fibrillary acidic protein (anti-GFAP) as a further indication of autoimmunity [19]. This autoimmune theory of autism was again reinforced by the observations of increased levels of urinary neopterin and biopterin, which were compatible with cellular immune activation [20]. Adding more weight to this argument was the observations by researchers at Washington University in St. Louis of autoantibodies to brain endothelium in children with symptoms of autism [21]. Connolly and associates then observed the association of brain directed sera autoimmune markers with autism, including elevations of brain derived neurotrophic factor (BDNF) as well as autoantibodies to BDNF [22]. Recently, researchers at the University of Cincinnati found a blood cytokine profile consistent with increased activation of both the Th1 and Th2 arms of the adaptive immune response, with Th2 predominance, but without the compensatory increase in the regulatory and anti-inflammatory cytokine Interleukin-10 (IL-10) [23].

Simultaneous to these immune investigations, other researchers were studying the link between dietary proteins and autism. Jyonouchi et al. observed that children with autism produced an excess of tumor necrosis factor alpha (TNF- α) in response to dietary proteins and to bacterial endotoxin-lipopolysaccharide [24,25]. Of further interest, in related research, others detected likely cross-reactivity of the wheat derived protein, gliadin, and Purkinje cell peptides [26]. The researchers noted an eight amino acid shared sequence that would be more than adequate to trigger immune cross-reactivity and hence, autoimmunity. This research would still fall short of a direct mechanism of peripheral activation of the immune system triggering neurodevelopmental disruption, but the dysregulated immune state of autism was becoming clearer.

As the immune aberrations in autism were being defined, another pathway of immunological research reported observations of a distinct inflammatory bowel disease [27] and other gastrointestinal disorders, including abnormal carbohydrate digestion and reflux esophagitis [28]. While this remains an emotionally charged debate in medicine secondary to issues surrounding purported measles, mumps, and rubella vaccine reactions [29], the observations of a panenteric bowel disease in a subset of children with autism are now well recorded in the medical literature [30]. The gastrointestinal inflammatory features of autism are now finding greater acceptance by diverse investigators [31–33].

More recently, focus has intensified on the immune findings in the autistic brain, which have been assisted by greater access to autism brain tissue samples, allowing researchers to define the immunological changes within the autism affected brain. Vargas et al. observed neuroglial activation and evidence of inflammation in both children and adults with autism [34]. Their observations included marked activation of microglia and astroglia. They also described cytokine profiling and indicated that macrophage chemoattractant protein-1 (MCP-1) and transforming growth factor beta-1, derived from neuroglia, were the most prevalent cytokines in autistic brain tissues. Furthermore, the cerebrospinal fluid (CSF) showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1 and interferon gamma. The findings suggest an immune-mediated brain inflammatory process. Intriguingly, researchers from the same institution were not able to reproduce similar findings in the CSF of a different population of autistic children in a later study [35]. This might be because the former study specifically examined brain tissue levels of immune activators, histological changes in both microglia and astroglial cells, and histologically documented perivascular neuroglial activation in the brain of children presenting with autism. Therefore, this inflammatory process might have been confined to the brain parenchyma. This observation is consistent with either astrocytic defense of the blood–brain barrier or autoimmune activation, and might correlate with the previously mentioned endovascular autoimmunity reported by Connolly et al. Most recently, Singer and colleagues, also at Johns Hopkins, found higher frequencies of serum anti-brain antibodies to specific brain regions in both children with autism and their siblings when compared to controls [36]. Taken together this data indicate significantly increased brain–immune interactions.

The favorable clinical responses reported after modification of the immune systems of autistic children support the underlying immune-mediated aspects of the disorder and also encourage interest in immunological interventions. Gupta et al. observed both immune dysregulation in this population and a favorable clinical response to treatment with human intravenous immunoglobulin (IVIG) [37]. Not all investigators have been able to reproduce this positive outcome from IVIG, but subpopulations of immune deficient children with autism have been reported to respond well to IVIG in the majority of cases [38].

Several other researchers have reported encouraging clinical improvements by the elimination of certain dietary proteins. As noted previously, these food antigens are now recognized as potent inducers of proinflammatory cytokines. A study in 1978 evaluated the effects of a carefully constructed elimination-reintroduction diet on disruptive and hyperactivity behavior in an 8-year-old autistic boy [39]. Results showed that foods such as wheat, corn, tomatoes, sugar, mushrooms, and dairy products were instrumental in producing behavioral disorders in this child. In another study, 36 Italian children with autism were evaluated for milk-related antibodies and response to milk elimination as compared to 20 control children [40]. The researchers noted higher antibody levels to milk proteins in the autistic population and a marked improvement in the behavioral symptoms of autistic patients after a two month milk elimination diet. Cade et al., at the University of Florida, reported a significant improvement in 81% of children with autism who went on a gluten and casein elimination diet [41]. A Norwegian cohort followed for four years with a similar diet also showed significant improvements in cognitive abilities and communicative skills [42].

Gupta reviewed the medical literature on various immunological interventions for autism including transfer factor, which has been shown to confer immunity; pentoxifylline, a phosphodiesterase inhibitor, which is known to have immunomodulatory effects; and IVIG, which has been shown to be beneficial in other neuroimmune and epileptic disorders [43]. As mentioned previously, Scifo et al. found that the therapeutic benefit of low dose naltrexone in children with autism was associated with a reduction in cytotoxic T-cells and the normalization of the CD4/CD8 ratio.

Typically, in refractory immune-activation disorders, especially autoimmune disorders, clinicians eventually attempt systemic corticosteroids in an attempt to down-regulate the immune

system. Little has been published regarding this intervention for autism; however, in an intriguing case-report, Shenoy et al. described a child who rapidly developed autism with the onset of autoimmune lymphoproliferative syndrome (ALPS) [44]. Low-dose steroid treatment resulted in rapid relief of the ALPS and eventual complete remission of the autism as well. Other reports also demonstrated significant improvements following corticosteroids in a child with pervasive development disorder [45] and with the use of pulsed oral steroids [46].

Evidence of elevated androgens in a subset of children with autism

A genetic link to the XY chromosome axis is lacking in autism, implying a likely hormonal vulnerability to certain environmental triggers. Data from several sources on the possible role of elevated androgens in aggressive behaviors, and possibly in the pathogenesis of autism, lends support to interventions that reduce androgenic activity as well. In addition to their published works on the subject [47], Geier and Geier have presented their unpublished clinical findings regarding leuprolide acetate, a synthetic non-peptide analog of naturally occurring gonadotropin-releasing hormone, as an intervention for children with concurrent autism and elevated androgens [48]. The parental and clinical observations they have made demonstrate improvements in some children and are intriguing. Other investigators have found leuprolide acetate beneficial in the management of troubling sexual behaviors in autism [49].

The potential use of spironolactone in autism

Given the large body of experimental and clinical observations regarding immunological and hormonal issues in autism, a preferred interventional agent would simultaneously address both issues. This agent might have the following properties: (1) downregulate the TNF- α response to provoking agents, (2) decrease MCP-1 and interferon-gamma in the brain with resultant decrease in glial activation and inflammation, (3) decrease inflammation in the gastrointestinal tract, (4) decrease androgenicity without interference of normal growth, (5) a favorable safety profile, and (6) low cost. Given these criteria, spironolactone would appear to possess these properties.

Cost and safety considerations

Spironolactone is an aldosterone antagonist available only by prescription. It is available as a generic medication and therefore has a relatively low cost (estimated cost 18–75 cents per day for typical doses, based on our informal review of internet based pharmacy prices). We have also had this medication compounded in pure form without excipients for less than \$25 per month. Spironolactone is generally known as a potassium-sparing diuretic and has had a lengthy period of observation in both children and adults. Its common and indicated uses are for congestive heart failure, hyperaldosteronism, edema associated with liver failure, nephrotic syndrome, hypertension, and hypokalemia (low serum potassium). It is considered safe for children when used in typical doses (1–3 mg/kg/day) [50], although the official FDA literature state that a safe range has not been established for children. Despite this fact, the oral LD50 of spironolactone is greater than 1000 mg/kg in mice, rats, and rabbits [51]. Further, a study in rats, dogs, and monkeys receiving spironolactone daily for up to two years, at doses frequently in excess of 100 times the recommended human dose, demonstrated no evidence to suggest that spironolactone is tumorigenic or carcinogenic [52].

History of spironolactone use as an anti-inflammatory agent

Spironolactone was first known to possess anti-inflammatory properties as early as 1961 [53]. However, that observation seemed to have gone largely unnoticed until the last few years. In a recent study, Japanese researchers looking for a reduction in cardiovascular risk factors related to inflammation found spironolactone to be the most potent anti-inflammatory medication they studied [54]. Specifically, it was found to potently reduce both TNF- α and MCP-1 in cultured human monocytes. These effects occurred at levels obtainable during routine oral administration of the medication. In a Danish population of rheumatoid arthritis patients (including juvenile idiopathic arthritis), a modest dose of 1–3 mg/kg/day resulted in a significant reduction of proinflammatory cytokines as well as decreased gene transcription for many regulators of inflammation [55]. Additionally in this study, incubated human whole blood, treated with spironolactone, demonstrated dramatically reduced interferon

gamma and substantially reduced TNF- α . None of the juvenile arthritis patients had to withdraw from this study, and eight out of nine children (mean age 12 years) responded favorably to spironolactone at a dose range of 2–3 mg/kg/day. One female patient had disruption of her menstrual cycle, a known side-effect. Further, in type 2 diabetics, spironolactone administration attenuated elevated MCP-1 levels and alleviated oxidative stress [56]. This is relevant because oxidative stress has been well described in some autistic children [57].

The use of spironolactone as a safe anti-androgen for acne, hirsutism, and precocious puberty

In an extensive literature analysis (Cochrane Review) of the use of spironolactone for hirsutism and acne in female patients, the reviewers concluded that 6 months of treatment with spironolactone (100 mg/day) was associated with a statistically significant subjective reduction in abnormal hair growth when compared to placebo [58]. Furthermore, spironolactone at a dose of 100 mg/day was superior to either finasteride or cyproterone acetate (other common anti-andro-

gens) and had little effects on DHEA, DHEA-S, or testosterone levels in the studies evaluated. This is because the mechanism of action of spironolactone occurs by attachment to dihydrotestosterone (DHT) receptors on cell surfaces, thereby inhibiting the binding of DHT to these receptors [59]. In an 1-year study of hirsutism in 12 prepubertal girls (mean age 6.9 years), spironolactone at doses up to 100 mg/day was noted to be effective, safe, and well tolerated [60]. Spironolactone is also commonly prescribed as an adjunct in the treatment of precocious puberty. In a six year study using spironolactone in 10 boys (ages 2.3–5.6 years) with precocious puberty, spironolactone was given with testolactone, an aromatase inhibitor that blocks the conversion of androgen to estrogen [61]. In this long term study, no serious side-effects were noted despite relatively high doses of spironolactone (average 5.7 mg/kg/day). Importantly, serum electrolytes remained in the normal range, which would indicate frequent blood electrolyte monitoring would likely be unnecessary. It was noted that significant reduction in aggressive behaviors occurred in two of the boys within six months of starting treatment. These studies demonstrate the safety and tolerability of spironolactone in prepubertal children over extended periods of time.

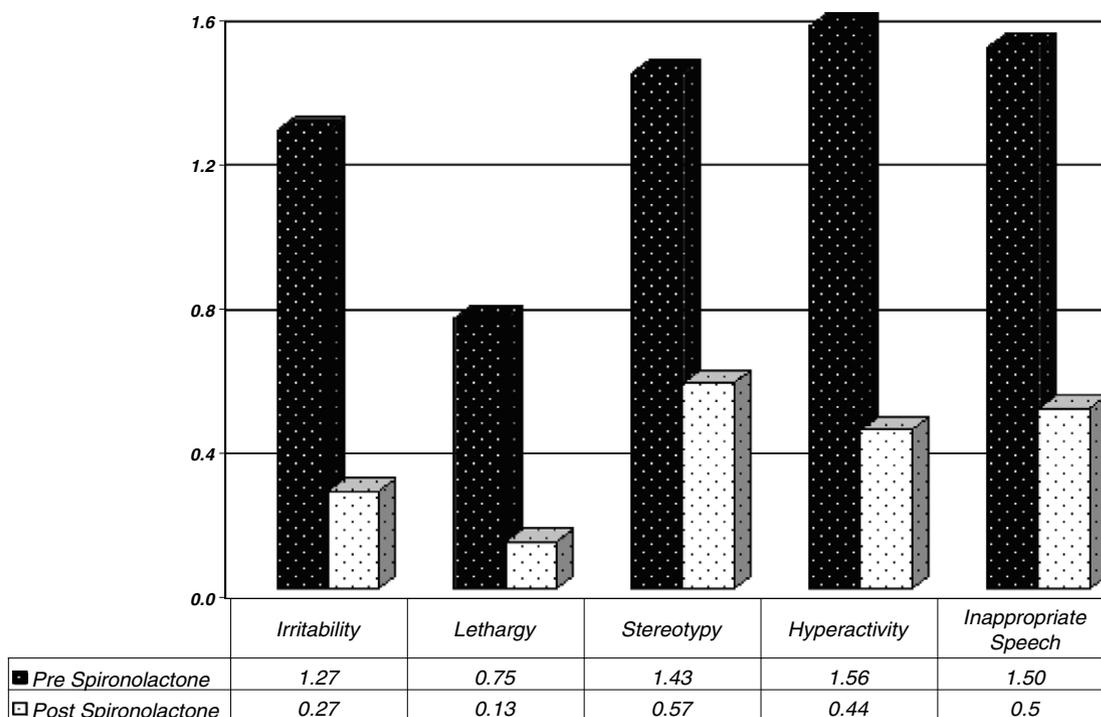


Figure 1 Changes in the ABC subset scores pre- and post-spironolactone at a dose of 2 mg/kg daily for 4 weeks.

Testing the hypothesis: early observations of the benefits of spironolactone in autism spectrum disorders

Other authors have termed the recent discovery of the immunologic benefits of spironolactone as its “renaissance” [62]. Our clinical experience with spironolactone and autism leads us to believe it might be successful in this population as well. While preliminary, we have collected a series of anecdotal observations where parents have noted substantial and rapid improvements including cognitive gains, diminished obsessive-compulsive behaviors, improved spontaneous socialization, reduced aggression, and improved sleep. As a more objective example, a 12-year-old boy with well-established autism, immune dysregulation, food allergies, and elevated testosterone levels demonstrated significant reductions in the severity and frequency of several aberrant symptoms within four weeks of spironolactone administration at a daily dose of 2 mg/kg. Specifically, administrations of the Aberrant Behavior Checklist (ABC) [63] by a licensed psychologist and Board Certified Behavior Analyst (DG) before and after the implementation of spironolactone indicated a 79% improvement in irritability, a 83% decrease in lethargy, a 60% reduction of stereotypy, a 72% reduction of hyperactivity, and a 67% decrease in inappropriate speech (see Fig. 1). In addition, pre- and post-administrations of the Peabody Picture Vocabulary Test III [64], also by the same

Table 2 Summary of the proposed effects of spironolactone on autism findings

Clinical finding	Autism finding	Effect of spironolactone
Interferon gamma	↑ [19]	↓ [55]
TNF- α	↑ [24,25]	↓ [54,55]
MCP-1	↑ [34]	↓ [54,56]
Inflammation	↑ [27,34]	↓ [53,55]
Oxidative stress	↑ [57]	↓ [62]
Testosterone effects	↑ ^a [5–7]	↓ [59]

^a Elevated in a subset of autistic individuals.

psychologist (DG), were scored independently by another psychologist employed by DG. These demonstrated a receptive language gain of 21 months in this same four week period, indicating an increase in vocabulary greater than one standard deviation at either age level. Lymphocyte counts as well as levels of immunoglobulins and testosterone measured immediately prior to treatment with spironolactone 100 mg daily (approximately 2 mg/kg/day) confirmed immune dysregulation and elevated testosterone (see Table 1). These results were similar to cellular immune parameters and testosterone levels assessed 1-year prior. Spironolactone would not be expected to significantly change testosterone levels, because as previously noted, its action is on receptor blockade; therefore, we have not requested post-spironolactone laboratory measurements.

Table 1 Levels of immunoglobulins and testosterone measured immediately prior to treatment with spironolactone

Measure	Patient level	Reference range ^a
IgG1	383 mg/dL	389–1372 mg/dL
IgG2	104 mg/dL	125–417 mg/dL
IgG3	24 mg/dL	28–135 mg/dL
IgG4	20 mg/dL	14–162 mg/dL
IgA	85 mg/dL	70–432 mg/dL
IgM	38 mg/dL	52–367 mg/dL
WBC	3.8 thous/mcL	4.5–13.5 thous/mcL
Total Lymphocyte Count	1204/CCM	1500–6500/CCM
Abs CD3	768/CCM	920–2200/CCM
Abs CD4	351/CCM	520–1440/CCM
Abs CD19 ⁺	173/CCM	200–820/CCM
Total testosterone	337 ng/dL	<260 ng/dL
% Free testosterone	0.53%	0.53–3.33%
Repeat total testosterone	369 ng/dL	<260 ng/dL
DHEA-S	92 mcg/dL	<235 mcg/dL

^a Reference ranges for immunoglobulins and lymphocyte subsets per Quest Diagnostics (lab where samples were analyzed). Testosterone related reference ranges from J Clin Invest 1974;53:819–28 and J Clin Endocrinol Metab 1973;36:1132–42.

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Conclusion

Spironolactone is a low-cost, easily available oral agent with a favorable safety profile, and with desirable immune and anti-inflammatory properties (see Table 2). Its secondary benefits as an anti-androgen might further enhance its appeal in autism, particularly in a definable subset of hyperandrogenic children. Additional research in controlled trials is now needed to further define the risks and benefits of spironolactone use in children with autism.

Acknowledgements

Statement of potential conflicts of interest: all of the authors, with the exception of D.G., have a child or children with autism or autism spectrum disorders. Three of the authors (J.B., S.S., and D.R.) are applying for a patent on the use of spironolactone in the treatment of autism. They have concurrently assigned any potential future financial interests in the patent to a non-profit organization involved in the treatment and research of autism. None of the authors or their families has financial ties to the non-profit organization. The authors were not compensated for their work on this paper or for the assignment of the patent application. Parental approval was obtained to publish the case report data in this paper.

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