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CASE REPORT

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Observed improvements in immune parameters and behavioral symptoms following low-dose IL-2 treatment in four autistic children with immune dysfunction

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Abstract

Background Autism spectrum disorder (ASD) is a complex neurodevelopmental condition for which there are no definitive medications targeting core symptoms. Growing evidence indicates that individuals with ASD exhibit immune dysfunction and evidence of peripheral and central inflammation. Immunotherapy interventions have shown promise in addressing this dysregulation. Notably, low-dose Interleukin-2 (Ld IL-2), an established treatment for certain autoimmune diseases, represents a potential therapeutic approach. Our study aims to investigate the association between Ld IL-2-induced immune modulation and behavioral improvements in children with ASD. This research may offer novel insights into potential treatment strategies for ASD.

Case presentation In our previously completed single-arm, self-controlled clinical study (data unpublished), 24 ASD children with immune abnormalities received Ld IL-2 treatment, all of whom showed varying degrees of symptom improvement. This paper presents case reports of four ASD children who demonstrated marked clinical improvement and substantial correction of immune imbalance following treatment. The average age of 4 participants was approximately 6 years old, comprising two boys and two girls. Assessments using the Childhood Autism Rating Scale(CARS), Aberrant Behavior Checklist (ABC), Autism Treatment Evaluation Checklist (ATEC), Krug's Autism Behavior Scale (CABS), Hospital Anxiety and Depression Scale (HADS), and Caregiver Strain Questionnaire (CGSQ) revealed substantial behavioral improvements and no adverse events. Improvements persisted for three months post-treatment, with notable progress in Speech/Language/Communication and body/health/behavior domains. According to caregivers, there has been a observed improvement in the subjects' sleep quality, accompanied by a gradual restoration of overall physical health. Immune testing in the four pediatric patients revealed a marked decrease in both the proportion of Type 1 cytotoxic T cells (Tc1) and the Tc1/Regulatory T cell ratio.

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Conclusions Our findings in these four cases describe an association between Ld IL-2 treatment and observed improvements in ASD symptoms, potentially linked to immune modulation.

Keywords Autism spectrum disorders, Low-dose IL-2, Immune dysfunction, Regulatory t cell, Cytokines

Background

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social interaction, communication, and repetitive behaviors [1]. The exact causes of ASD remain unclear, but factors such as genetics, environment, and immune system abnormalities are believed to contribute to its abnormal neurological development [2]. The prevalence of ASD has been increasing, affecting approximately 1 in 36 children aged 8 years, with boys being 3.8 times more likely to be diagnosed than girls [3]. Current treatment strategies primarily involve behavioral interventions, although medications like risperidone and aripiprazole, approved for managing behavioral symptoms, do not address core ASD features and come with varying side effects [4]. Many individuals with ASD, around 70%, also contend with anxiety, hyperactivity, insomnia, and difficulties in social interactions and repetitive behaviors. Additionally, about half of ASD patients exhibit mild to severe intellectual disability [2]. Given ASD's diverse manifestations, there is a growing emphasis on classifying patients more precisely and developing targeted treatments. Recent research suggests that immune system dysregulation, particularly reduced levels of regulatory T cells (Tregs), may be prevalent in some individuals with ASD [5, 6]. Clinical trials investigating immunotherapy have shown promising results [7–9]. Interleukin-2 (IL-2), a cytokine with diverse biological effects, modulates lymphocyte activity by interacting with IL-2 receptors on various immune cells, thereby influencing immune response and cytokine production [10, 11]. In most autoimmune diseases (AIDs) and inflammatory diseases, there is a effector T cells (Teff)/Treg imbalance [12, 13]. Immune imbalance refers to abnormal ratios of Teffs-including T helper type 1 (Th1), type 2 (Th2), type 17 (Th17), and cytotoxic T type 1 (Tc1) cells-to Tregs, specifically values exceeding reference ranges established in healthy populations. However, Low-dose IL-2 (Ld IL-2) treatment can preferentially expand the Treg population, thereby re-establishing immune tolerance [14]. Ld IL-2 enhances the level and function of Treg cells, and inhibits the inflammatory response caused by effector Teff cells such as Tfh and Th17 cells, thereby improving AIDs [15, 16]. Ld IL-2 has been effective in ameliorating symptoms and immune dysfunction in AIDs [17]. Elevated levels of pro-inflammatory cytokines, including IL-6, IL-17 A, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), have been observed in some children with ASD. Some studies suggest a strong association between ASD and AIDs [18–20]. Therefore, this exploratory case report examines the effects of Ld IL-2 treatment on core ASD symptoms and the quality of life for four children with ASD with immune dysregulation.

Case report

These four participants were selected from a registered clinical trial (ChiCTR2000040836), which investigates Ld IL-2 in ASD children with immune dysregulation (data unpublished), and received ethical approval (Review-PJ-2020-102). All participants were screened according to the inclusion/exclusion criteria before enrollment.The inclusion and exclusion criteria were based on data from a previous study [21].

Subject 1 is a 6-year-old girl born at full term with a birth weight of 2.95 kg and no history of perinatal asphyxia or allergies. During the neonatal period, she had elevated serum bilirubin levels (17–18 mg/dL). Developmentally, she achieved normal milestones before the age of 1, including eye contact, visual tracking, and laughter, without nystagmus or athetosis. However, she displayed stereotypic movements and sleep disturbances. Her Electroencephalogram (EEG) findings were normal, and her hearing was within the normal range. She was diagnosed with ASD according to The diagnostic and statistical manual of mental disorders, 5th Edition (DSM-5) criteria (https://psycnet.apa.org/record/2013-14907-000) by licensed medical doctors (MDs). Presently, she weighs 17 kg and measures 117 cm in height. Subject 1 experiences frequent constipation and has been undergoing rehabilitation for nearly five years. She exhibits occasional responses but does not speak, cannot perform tasks upon command, and requires assistance with basic daily activities. She has poor sleep quality and experiences walking instability leading to frequent falls. Subject 1 had received acupuncture previously but had not undergone medical therapy in the preceding six months. Written informed consent was obtained from the parents or legal guardians of Patient 1 for their child's participation in the study and the publication of anonymized case details. Reasons for inclusion: Patient 1 exhibited an elevated proportion of Th1, Th2, and Tc1 cells, with an imbalance between Th1/Treg ratios (≥ 0.63) and Th2/Treg ratios (≥ 0.17) Among them, the proportion of Tc1 cells (15.01%) was markedly higher than one of the inclusion criteria based on our previous data (the proportion of Tc1 cells ≥ 7.39%).

Subject 2 is a 6-year-old boy born at term with a birth weight of 3.2 kg and no history of birth asphyxia or

allergies. He frequently experiences colds. Before the age of 2, he exhibited delayed language development, lack of responsiveness, and hyperactivity. At 2 years and 4 months old, hearing tests and cranial MRI results were normal, while EEG showed abnormalities suggestive of developmental delay. He was diagnosed with ASD by licensed MDs according to DSM-5 criteria, and for the past 4 years, he has received rehabilitation therapy, showing some improvement in language skills and mood. Currently, he weighs 20.5 kg and measures 115.5 cm in height. Subject 2 can perform self-care activities but requires assistance with eating, and he experiences poor sleep quality. His parents are in good health, and he has one elder sister who is developing typically. Written informed consent was obtained from the parents or legal guardians of Patient 2 for their child's participation in the study and the publication of anonymized case details. Reasons for inclusion: In Patient 2, the proportion of Treg cells (0.4%) was too low (one of our inclusion criteria was that the number of Treg cells ≤ 2.21%). and an imbalance was also observed between Th1/Treg and Th2/Treg ratios.

Subject 3 is a 4-year-old girl born at full term with a birth weight of 2.75 kg and no history of birth asphyxia or allergies. At 1 year of age, she was hospitalized due to rotavirus infection, after which she began exhibiting preferences in diet, along with delays in language, fine motor skills, and cognitive development. She also showed emotional irritability, insecurity, a tendency to play with hands, stereotyped movements, and difficulty maintaining eye contact. Subject 3 struggles to follow commands and experiences poor sleep, though she can produce simple pronunciations. A diagnosis of ASD was made according to DSM-5 criteria by licensed MDs involved in this study. At the time of assessment, she weighed 11.5 kg and measured 94.5 cm in height. Her father is in good health, while her mother has allergic rhinitis. Written informed consent for participation in the study and for the publication of anonymized case details was obtained from the parents or legal guardians of Patient 3. Reasons for inclusion: Patient 3 showed a marked increase in the proportion of Tc1 cells (15.07%).

Subject 4 is an 8-year-old boy born at term with a birth weight of 3.25 kg and no history of neonatal intensive care unit admission or allergies. He began vocalizing "papa, mama" at 1 year of age but later exhibited frequent falls, reluctance to speak, reduced eye contact, and a glazed expression by 18 months. At 3 years old, he underwent cerebrospinal fluid analysis, which confirmed rubella viral encephalitis (IgM). EEG findings were abnormal, while cranial MRI showed no anomalies. The treatment regimen included antiviral therapy, intravenous fluids, interferon injections, oral antiviral medications, hyperbaric oxygen therapy, corticosteroid treatment, and oral Chinese medicine. Following this comprehensive treatment approach, the patient demonstrated recovery. Currently, he independently performs activities of daily living and experiences restful sleep. ASD was diagnosed according to DSM-5 criteria by licensed MDs involved in this study. He weighed 27 kg and measured 136 cm in height. Subject 4's family has no history of psychiatric disorders; his sister, father, and mother are in good health. Written informed consent for participation in the study and for the publication of anonymized case details was obtained from the parents or legal guardians of Patient 4. Reasons for inclusion: Patient 4 had a reduced proportion of Treg cells (2.21%) and elevated Th2/Treg (0.25). A summary of basic information can be found in Table 1.

Table 1 Social demographic data

	Patient 1	Patient 2	Patient 3	Patient 4
Gender	female	male	female	male
Age	6	6	4	8
Weight (kg)	17	20.5	11.5	27
Height (cm)	117	115.5	94.5	136
Ethnic groups	Han	Yi ethnic group	Han	Han
siblings	0	1	0	1
siblings' gender	-	female	-	female
Whether siblings are typically developing	-	Yes	-	Yes

Before treatment initiation, each patient underwent routine blood tests and a standard electrocardiogram to establish baseline health and assess eligibility for therapy. The treatment regimen comprised three cycles of subcutaneous injections of Ld IL-2 (Shandong Quanqi, 500,000 IU/bottle) at a dose of 16,000 IU/kg. Injections were alternated between the left and right arms during each cycle. Each cycle included seven injections of Ld IL-2 (subcutaneously every other day) followed by a 14-day rest period, ensuring sterile administration. Patients were evaluated using standardized assessments before treatment, after treatment, and at a three-month follow-up post-treatment. Assessment tools included the Childhood Autism Rating Scale (CARS), Aberrant Behavior Checklist (ABC), Autism Treatment Evaluation Checklist (ATEC), and Krug's Autism Behavior Scale (CABS). CARS is commonly used in the screening, diagnosis, and evaluation of treatment outcomes for children with ASD. This scale involves a comprehensive evaluation across 15 domains, with each domain assessed by trained professionals. The ABC is completed by caregivers and includes five key areas: sensory processing, communication, physical movement, language, and self-care. To assess the effectiveness of treatment, the ATEC is utilized, evaluating four domains: social behavior, language, perception, and behavior. Like the ABC, the ATEC is also filled out by

caregivers. The CABS is another tool employed to assess specific behavioral characteristics associated with ASD, focusing on social interaction, communication skills, interests, and repetitive behaviors. Caregiver outcomes were measured using the Hospital Anxiety and Depression Scale (HADS) and Caregiver Strain Questionnaire (CGSQ). Additionally, venous blood samples were collected before and after treatment for quantitative cytokine experiments. Peripheral blood mononuclear cells were isolated and analyzed for immune cell populations and serum cytokine levels, including Treg cells, T helper cell subsets (Th1, Th2, Th17), T follicular helper (Tfh), Tc1, and various cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, IL-12P70, IL-1β, TNF-α, TNF-β, IL-17 A, IL-17 F, IL-22, TGF-β). The units of cytokines are pg/ml.

Throughout the treatment period, all four patients underwent behavioral interventions at various rehabilitation facilities. We assessed patients' scale scores and immune biomarkers before and after treatment. Following LdIL-2 treatment, improvements were noted in CARS, ABC, ATEC, and CABS scores for all patients (Table 2). Patient 1 showed notable decreases in CARS, ABC, ATEC, and CABS scores. These reductions were maintained at the 3-month follow-up, although ABC and CABS scores showed some increase. At the end of the treatment course, caregivers' CGSQ scores and HADS' depression scores increased, while HADS' anxiety scores decreased. At follow-up, both CGSQ scores and anxiety scores decreased compared to pre-treatment levels, but depression scores remained higher than pre-treatment levels. Patient 2 exhibited substantial decreases in CARS, ABC, ATEC, and CABS scores. Caregivers' CGSQ scores showed a notable increase, while depression and anxiety scores decreased. However, follow-up data were unavailable due to family relocation. Patient 3 demonstrated substantial decreases in CARS, ABC, ATEC, and CABS scores, with caregivers showing decreased CGSQ and HADS scores. At follow-up, the therapeutic effects of Ld IL-2 for Patient 3 persisted. However, while caregivers' HADS anxiety scores decreased, the depression scores increased. Patient 4 also experienced marked decreases in CARS, ABC, ATEC, and CABS scores, with caregivers initially showing decreased CGSQ and HADS scores. However, caregivers' CGSQ and deression scores rose during the follow-up period. In short, caregivers reported a decrease in anxiety scores, although improvements in depression were not substantial. Radar charts (Figs. 1 and 2) depicted changes in CARS and ATEC scores pre- and post-treatment. During treatment, Patients 1 and 2 experienced brief hospitalizations due to fever and cold symptoms lasting over a week. Caregivers reported improved sleep quality, stabilized mood, and reduced hyperactivity in Patients 1, 2, and 3 post-treatment. Patient 2's caregivers noted enhanced adherence to traffic rules and improved danger perception following treatment with Ld IL-2.

For comparison, Table 3 includes the immunization results for a healthy child [21] from a previous study. Immune response patterns varied among patient post-treatment. Patient 1 exhibited reduced Treg cells and an increased Th1/Th2 ratio, whereas Patients 2, 3, and 4 showed elevated Treg cell counts and a decreased Th1/Th2 ratio. Patient 3 additionally demonstrated an increased Th2/Treg ratio. Tc1 cells and the Tc1/Treg ratio were significantly reduced in all patients, while IL-12P70 levels showed a mild reduction (Table 3). Due to the small cohort size, statistical correlation analyses between immune markers and behavioral scores were not performed. Observed trends (e.g., Treg increase with ATEC reduction) are descriptive and require validation in larger trials.

Discussion and conclusions

Currently, the treatment landscape for ASD predominantly revolves around behavioral interventions complemented by pharmacotherapies. Despite explorations into

Table 2 Scores on scales related to the situation of ASD patients and caregivers

		CARS	ABC	ATEC	CABS	CGSQ	HADS*
Patient1	Before treatment	53	79	118	25	52	6/0
	After treatment	44	47	106	11	62	4/4
	Follow up	36	53	49	12	49	2/3
Patient2	Before treatment	46	88	97	18	88	4/12
	After treatment	36	46	71	14	96	2/10
	Lost to follow-up	-	-	-	-	-	-
Patient3	Before treatment	43	78	99	25	58	6/9
	After treatment	41	38	81	20	36	6/8
	Follow up	41	34	86	23	31	2/14
Patient4	Before treatment	34	63	49	24	44	4/5
	After treatment	27	58	31	19	33	3/0
	Follow up	24	37	24	13	47	2/4

* HADS part include anxiety and depression, tables show as follows: anxiety/depression

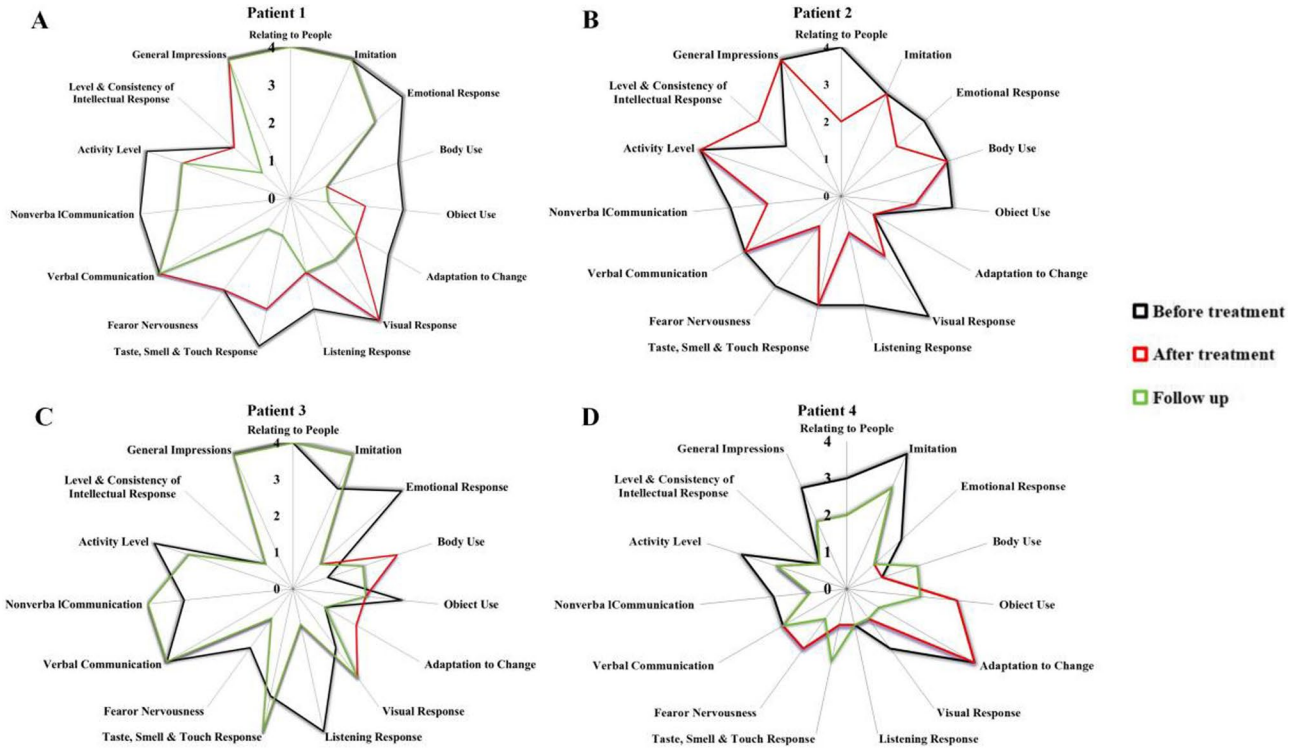

Fig. 1 Changes in the 15 dimensions of the CARS scale in four ASD patients

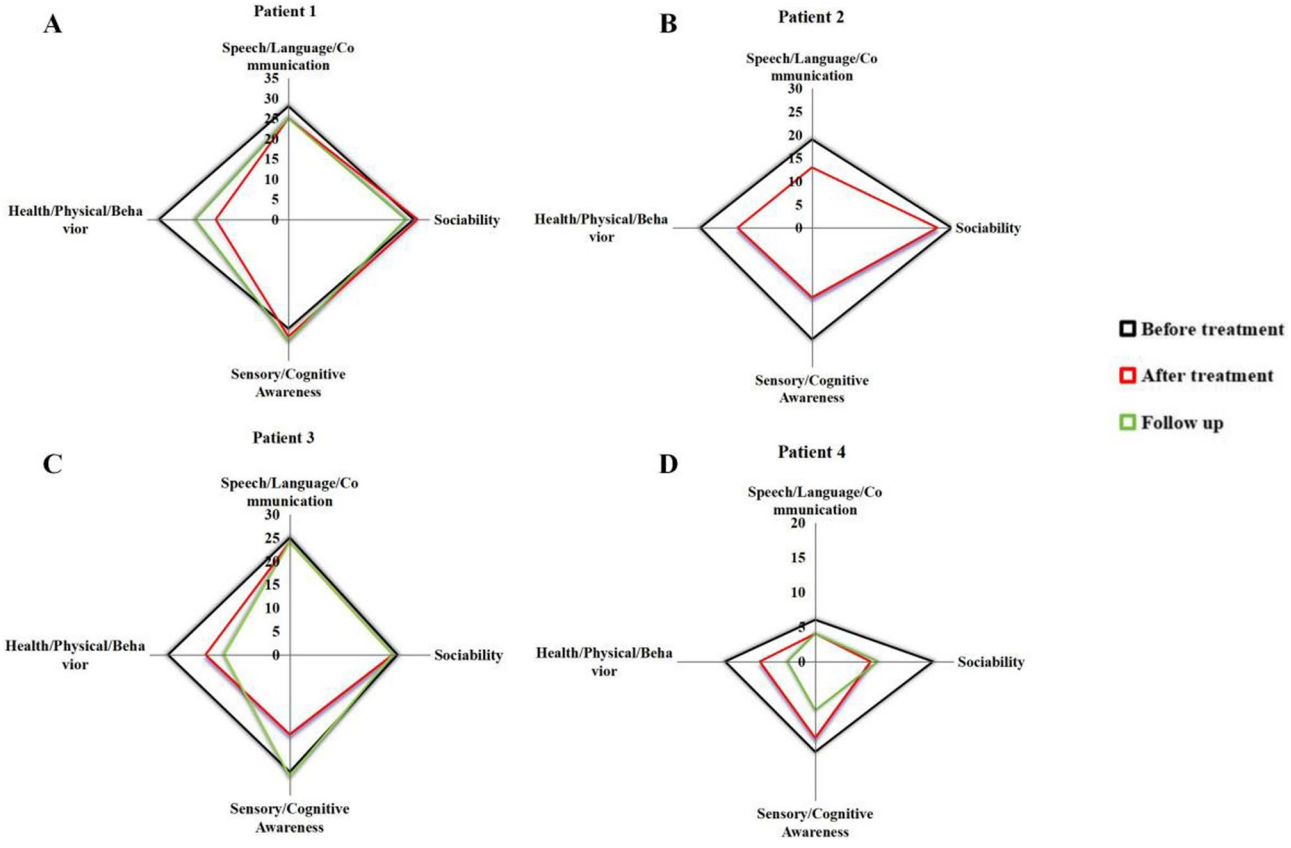

Fig. 2 Changes in the 4 dimensions of the ATEC scale in four ASD patients

Table 3 Table 3 observed levels of T cell subsets and cytokines before and after treatment

	a healthy child	Patient 1		Patient 2		Patient 3		Patient 4	
		Before	After	Before	After	Before	After	Before	After
Treg	5.53	3.10	2.75	0.40	4.93	2.91	3.77	2.21	2.67
Tfh	-	0.03	0.29	0.26	0.28	0.29	0.46	1.56	0.48
Th1	1.34	2.50	2.47	1.39	1.71	1.53	2.13	0.56	0.26
Th2	0.94	0.85	0.32	0.39	0.64	0.27	0.60	0.56	0.57
Th17	0.62	0.08	0.11	0.04	0.08	0.02	0.32	0.21	0.68
Tc1	0.14	15.01	8.43	6.20	4.53	15.07	0.31	1.79	0.35
Th1/Treg	0.24	0.81	0.90	3.48	0.35	0.53	0.56	0.25	0.10
Th2/Treg	0.17	0.27	0.12	0.98	0.13	0.09	0.16	0.25	0.21
Tfh/Treg	-	0.01	0.11	0.65	0.06	0.10	0.12	0.71	0.18
Th17/Treg	0.11	0.03	0.04	0.10	0.02	0.01	0.08	0.10	0.25
Tc1/Treg	0.03	4.84	3.07	15.5	0.92	5.18	0.08	0.81	0.13
Th1/Th2	1.43	2.94	7.72	3.56	2.67	5.67	3.55	1.00	0.46
IL-4	2.01	3.52	7.37	2.10	1.95	3.66	2.86	2.51	3.3
IL-5	3.75	2.80	3.10	2.50	1.90	1.33	1.73	1.61	2.64
IL-6	5.87	4.03	6.59	3.99	4.59	4.34	4.03	4.60	3.59
IL-8	0.80	7.92	9.51	3.32	2.75	3.53	4.01	4.50	8.71
IL-10	5.46	6.23	6.84	4.18	3.76	4.44	4.35	4.26	4.87
IL-12P70	2.67	4.94	4.53	3.93	3.14	5.12	5.03	3.92	3.27
IL-1β	0.98	2.27	3.49	2.37	2.06	2.55	2.39	1.91	1.73
IL-2	8.09	3.53	3.84	2.67	2.73	2.82	2.76	3.09	1.48
IFN-γ	3.54	6.58	9.74	4.02	1.52	6.98	3.37	5.53	4.40
TNF-α	2.48	8.54	13.78	6.24	4.17	3.42	5.28	6.83	6.77
TNF-β	-	6.38	4.2	4.45	3.47	3.31	3.29	3.98	4.27
IL-17 A	1.03	5.84	10.41	6.47	5.19	6.86	6.31	4.99	5.36
IL-17 F	3.17	5.14	2.10	3.45	3.64	1.01	0.73	4.17	3.91
IL-22	0.99	2.31	1.65	0.76	0.28	0.62	0.78	1.87	1.25
TGF-β	4327.03	1762.86	877.17	1741.16	1051.35	1308.5	1948.92	205.12	1597.07

therapies such as intravenous immunoglobulin therapy [22] and stem cell therapy [23], none have shown substantial efficacy in alleviating core ASD symptoms. Given the prevalent immune dysregulation observed in ASD, particularly the number and function of Treg cells are particularly reduced, Ld IL-2 therapy has emerged as a promising avenue [24]. A number of clinical trials showed that Ld IL-2 is safe and effective treatment of AIDs, including systemic lupus erythematosus [25] and type 1 diabetes [26], primary sjogren's syndrome [27] and so on. A clinic trials showed that Ld IL-2 treatment of major depressive disorder and bipolar disorder of depression in patients has safety and clinical efficacy, and treatment that enhances the T cell system may be a way to correct the immune inflammatory abnormalities associated with mood disorders and enhance the antidepressant response [28]. Our study, consistent with international and domestic research, identifies immune system dysfunctions in both peripheral and central systems of children with ASD [21, 29–32]. Immune imbalance and inflammatory lesions are likely to participate in and aggravate ASD lesions, and improving these immune imbalances may be a potential treatment.

In this study, four children diagnosed with ASD with immune dysregulation showed meaningful clinical improvements following Ld IL-2 treatment, as assessed by healthcare professionals and caregivers. Standardized scales including CARS, ABC, ATEC, and CABS indicated notable reductions across multiple domains. Specifically, improvements were most pronounced in the domains of 'Emotional Response,' 'Obiect Use,' and 'Activity Level,' as measured by the CARS scale. ASD is characterized by restricted interests in emotional circuits and repetitive behaviors, often leading to exaggerated subjective experiences and difficulty in appropriately responding to emotions [33, 34]. Dysfunction in emotional processing involving areas such as the cerebral cortex, hippocampus, and amygdala is evident from autopsy findings, suggesting structural abnormalities that impair normal emotional feedback mechanisms [35]. ASD often manifests with abnormal hyperactivity and frequently co-occurs with Attention Deficit/Hyperactivity Disorder [36]. The observed behavioral improvements, particularly in emotional response and communication, warrant further investigation into whether Ld IL-2 might modulate neuro-immune interactions relevant to ASD symptoms. However, direct evidence of central nervous system

changes was not obtained in this study. Following Ld IL-2 therapy, meaningful improvements were observed in the 'Speech/Language/Communication' and 'Health/Physical/Behavior' domains of ATEC. Many ASD patients experience impairments in language and communication skills, such as speech delays, limited vocabulary, and difficulty with logical reasoning, profoundly impacting their quality of life [37]. Health-related issues, including sleep disturbances and dietary irregularities, are prevalent among ASD patients and significantly affect their well-being and that of their families. Caregiver reports suggest that Ld IL-2 treatment improves sleep quality and immune function in ASD patients. The mechanism underlying these improvements may involve modulation of immune dysregulation observed in ASD.

Evidence suggests that the dysregulation of Th1- and Th2-like cytokines in children with ASD may contribute to its pathogenesis [38]. The abnormal proportions of Th1, Th2, Th17, Tc1 and Treg cells, along with changes in related transcription factors and signal transduction pathways, can result in immune imbalance [5]. Several studies indicate that modulating this immune imbalance can effectively ameliorate core ASD symptoms. In our study, we evaluated immune parameters in children with ASD before and after treatment with Ld IL-2. Except for subject 1, the other three ASD children showed an increased proportion of Treg cells after Ld IL-2 treatment. along with a decrease in the proportions of Tc1 cells, and ratios of Th1/Th2, Th2/Treg, Tc1/Treg, and IL-12p70 levels. Elevated IL-12P70 levels have been observed in ASD patients and are associated with repetitive behaviors. Although limited by the sample size and no statistical tests were conducted, we observed that when the proportion of Treg increased, the total ATEC score tended to decrease (patients 2,3,4), and patients with higher IL-8 levels (such as patient 1) showed a more pronounced decrease in the ABC score. These findings suggest a pronounced association between T cell subgroups and behavioral changes, indicating a potential link. IL-8, a proinflammatory factor, shows a marked increase in children with ASD, correlating highly with parental reports and suggesting it merits further investigation as a potential biomarker [39, 40].

This study observed heterogeneity in TGF-β changes following Ld IL-2 treatment: levels increased in Patients 3–4 but decreased in Patients 1–2. This phenomenon necessitates an integrated interpretation based on TGF-β's biological properties and therapeutic mechanisms. It's anti-inflammatory effects: Promotes Treg differentiation and suppresses inflammation (e.g., Th17 activity) [41]. The post-treatment decline in TGF-β (Patients 1–2) may reflect attenuated inflammation, reducing the need for high-level immunosuppressive feedback. Additionally, compartmentalized consumption of TGF-β likely occurs: ASD patients frequently exhibit gut barrier disruption and neuroinflammation [42]– [43]. After Ld IL-2 therapy, Tregs migrate to inflammatory sites and locally release TGF-β, diminishing serum levels while enhancing regional immunomodulation. Pretreatment immune systems in chronic inflammation (e.g., high Tc1, low Treg) required sustained TGF-β secretion to counter inflammation-albeit insufficiently. The heterogeneity also arises from baseline immunological differences among patients (e.g., Treg/Tc1 ratios). Critically, reduced TGF-β in Patients 1–2 may represents a physiological adaptation during immune reconstitution, aligning with overall clinical improvement and underscoring the therapy's multifaceted efficacy. But these interpretations are limited by the small sample size and requires validation in larger studies.

In conclusion, this case report describes observed improvements in behavioral symptoms and immune parameters in four children with ASD and immune dysregulation following Ld IL-2 treatment. Given the exploratory nature of this report and the small sample size ($n = 4$), these findings must be interpreted cautiously and warrant further investigation in larger cohorts. Nevertheless, these preliminary findings support the rationale for further investigation into Ld IL-2 as a potential immunomodulatory approach for ASD individuals with specific immune profiles. Future validation critically requires: large-scale, randomized, placebo-controlled clinical trials that account for concurrent behavioral interventions; absolute lymphocyte quantification to refine immune profiling; and mechanistic studies dissecting IL-2-mediated neuro-immune crosstalk. Addressing these priorities is essential to determine whether precision immunotherapy could be a viable strategy for this immune-dysregulated ASD subgroup.

Abbreviations

ABC	Aberrant behavior checklist
ASD	Autism spectrum disorder
ATEC	Autism treatment evaluation checklist
CABS	Krug's autism behavior scale
CARS	Childhood autism rating scale
CGSQ	Caregiver strain questionnaire
DSM-5	Diagnostic and statistical manual of mental disorders, 5th edition
EEG	Electroencephalogram
HADS	Hospital anxiety and depression scale
IFN-γ	Interferon-gama
IL-1β	Interleukin-1 beta
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12P70	Interleukin-12P70
IL-17A	Interleukin-17A
IL-22	Interleukin-22
Ld IL-2	Low-dose IL-2
MDs	Medical doctors
Tc1	Cytotoxic T 1

Tfh	T follicular helper
TGF-β	Transforming growth factor-beta
Th1	T-helper 1
Th2	T-helper 2
TNF-α	Tumor necrosis factor-α
TNF-β	Tumor necrosis factor-beta
Treg	Regulatory T cell

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12888-025-07389-9.

Supplementary Material 1.

Supplementary Material 2.

Authors' contributions

Huiling Chen wrote the article and processed the images. Meng Li, Penghao Yao, and Huiling Chen documented the entire process of tests and experiments conducted on different subjects, from pre-treatment through to follow-up. Zuqing Nie performed the detection of T cell subsets using flow cytometry. Yan Zhang and Li Xu managed subject enrollment and conducted scale evaluations. Chen Shen and Xinyi Xu conducted data statistics, analyses, and provided assistance with flow cytometry. Juli Huang and Yan Liu are primarily responsible for administering drugs and verifying drug protocols. Zhiwei Li and Jie Wen assisted in processing blood samples from the subjects. Xia Cao and Lin Zhao reviewed the manuscript comprehensively and contributed throughout the study, with Lin Zhao also overseeing subject enrollment.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study, conducted at the Second Affiliated Hospital of Kunming Medical University (ID: 2020ynlc001), received ethical approval from the Medical Ethics Committee of the Second Affiliated Hospital of Kunming Medical University (Approval ID: Review-PJ-2020-102) on November 9 2020, in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants and their families. The study was registered as a clinical trial with the Chinese Clinical Trial Registry (ChiCTR ID: ChiCTR2000040836) on December 11, 2020, accessible via the ChiCTR website (http://www.chictr.org.cn/).

Consent for publication

Given that the case reports involve underage children, written informed consent was obtained from the parents or legal guardians of each patient for the publication of this case report.

Competing interests

The authors declare no competing interests.

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References

1. Volkmar FR, McPartland JC. From Kanner to DSM-5: autism as an evolving diagnostic concept. ANNU REV CLIN PSYCHO. 2014;10:193–212.
2. Lai MC, Lombardo MV, Baron-Cohen S. Autism LANCET. 2014;383(9920):896–910.
3. Maenner MJ, Warren Z, Williams AR, Amoakohene E, Bakian AV, Bilder DA, Durkin MS, Fitzgerald RT, Furnier SM, Hughes MM, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 Years - autism and developmental disabilities monitoring network, 11 sites, united states, 2020. MMWR SURVEILL SUMM. 2023;72(2):1–14.
4. Hirota T, King BH. Autism spectrum disorder: A review. JAMA-J AM MED ASSOC. 2023;329(2):157–68.
5. Ahmad SF, Zoheir K, Ansari MA, Nadeem A, Bakheet SA, Al-Ayadhi LY, Alzahrani MZ, Al-Shabanah OA, Al-Harbi MM, Attia SM. Dysregulation of Th1, Th2, Th17, and T regulatory cell-related transcription factor signaling in children with autism. MOL NEUROBIOL. 2017;54(6):4390–400.
6. De Giacomo A, Gargano CD, Simone M, Petruzzelli MG, Pedaci C, Giambersio D, Margari L, Ruggieri M. B and T immunoregulation: A new insight of B regulatory lymphocytes in autism spectrum disorder. FRONT NEUROSCI-SWITZ. 2021;15:732611.
7. Nestor MW, Phillips AW, Artimovich E, Nestor JE, Hussman JP, Blatt GJ. Human inducible pluripotent stem cells and autism spectrum disorder: emerging technologies. AUTISM RES. 2016;9(5):513–35.
8. Melamed IR, Heffron M, Testori A, Lipe K. A pilot study of high-dose intravenous Immunoglobulin 5% for autism: impact on autism spectrum and markers of neuroinflammation. AUTISM RES. 2018;11(3):421–33.
9. Chen DT, Chang JP, Cheng SW, Chang HC, Hsu JH, Chang HH, Chiu WC, Su KP. Kawasaki disease in childhood and psychiatric disorders: A population-based case-control prospective study in Taiwan. BRAIN BEHAV IMMUN. 2022;100:105–11.
10. Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. Immunity. 2010;33(2):153–65.
11. Abbas AK, Trotta E, Marson RSD, Bluestone A. JA: Revisiting IL-2: biology and therapeutic prospects. SCI IMMUNOL. 2018, 3(25):eaat1482.
12. Savage PA, Klawon D, Miller CH. Regulatory T cell development. ANNU REV IMMUNOL. 2020;38:421–53.
13. Dikiy S, Rudensky AY. Principles of regulatory T cell function. Immunity. 2023;56(2):240–55.
14. Klatzmann D, Abbas AK. The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. NAT REV IMMUNOL. 2015;15(5):283–94.
15. Graßhoff H, Comdühr S, Monne LR, Müller A, Lamprecht P, Riemekasten G, Humrich JY. Low-Dose IL-2 therapy in autoimmune and rheumatic diseases. FRONT IMMUNOL. 2021;12:648408.
16. He J, Zhang X, Wei Y, Sun X, Chen Y, Deng J, Jin Y, Gan Y, Hu X, Jia R, et al. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. NAT MED. 2016;22(9):991–3.
17. Collison J. Low-dose IL-2 therapy for autoimmune diseases. NAT REV RHEUMATOL. 2019;15(1):2.
18. Roe K. Autism spectrum disorder initiation by Inflammation-Facilitated neurotoxin transport. NEUROCHEM RES. 2022;47(5):1150–65.
19. Wu S, Ding Y, Wu F, Li R, Xie G, Hou J, Mao P. Family history of autoimmune diseases is associated with an increased risk of autism in children: A systematic review and meta-analysis. NEUROSCI BIOBEHAV R. 2015;55:322–32.
20. Than U, Nguyen LT, Nguyen PH, Nguyen XH, Trinh DP, Hoang DH, Nguyen P, Dang VD. Inflammatory mediators drive neuroinflammation in autism spectrum disorder and cerebral palsy. SCI REP-UK. 2023;13(1):22587.
21. Nie Z, Han D, Zhang K, Li M, Kwon H, Im S, Xu L, Yang J, Li Z, Huang X, et al. TH1/Treg ratio May be a marker of autism in children with immune dysfunction. RES AUTISM SPECT DIS. 2023;101:102085.
22. Connery K, Tippett M, Delhey LM, Rose S, Slattery JC, Kahler SG, Hahn J, Kruger U, Cunningham MW, Shimasaki C, et al. Intravenous Immunoglobulin for the treatment of autoimmune encephalopathy in children with autism. TRANSL PSYCHIAT. 2018;8(1):148.
23. Villarreal-Martínez L, González-Martínez G, Sáenz-Flores M, Bautista-Gómez AJ, González-Martínez A, Ortiz-Castillo M, Robles-Sáenz DA, Garza-López E. Stem cell therapy in the treatment of patients with autism spectrum

disorder: a systematic review and Meta-analysis. STEM CELL REV REP. 2022;18(1):155–64.
24. Zhang R, Zhao Y, Chen X, Zhuang Z, Li X, Shen E. Low-dose IL-2 therapy in autoimmune diseases: an update review. INT REV IMMUNOL. 2024;43(3):113–37.
25. He J, Zhang R, Shao M, Zhao X, Miao M, Chen J, Liu J, Zhang X, Zhang X, Jin Y, et al. Efficacy and safety of low-dose IL-2 in the treatment of systemic lupus erythematosus: a randomised, double-blind, placebo-controlled trial. ANN RHEUM DIS. 2020;79(1):141–9.
26. Zhang JY, Hamey F, Trzupek D, Mickunas M, Lee M, Godfrey L, Yang J, Pekalski ML, Kennet J, Waldron-Lynch F, et al. Low-dose IL-2 reduces IL-21(+) T cell frequency and induces anti-inflammatory gene expression in type 1 diabetes. NAT COMMUN. 2022;13(1):7324.
27. Miao M, Hao Z, Guo Y, Zhang X, Zhang S, Luo J, Zhao X, Zhang C, Liu X, Wu X, et al. Short-term and low-dose IL-2 therapy restores the Th17/Treg balance in the peripheral blood of patients with primary sjögren's syndrome. ANN RHEUM DIS. 2018;77(12):1838–40.
28. Poletti S, Zanardi R, Mandelli A, Aggio V, Finardi A, Lorenzi C, Borsellino G, Carminati M, Manfredi E, Tomasi E, et al. Low-dose Interleukin 2 antidepressant potentiation in unipolar and bipolar depression: safety, efficacy, and immunological biomarkers. BRAIN BEHAV IMMUN. 2024;118:52–68.
29. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, Ji L, Brown T, Malik M. Elevated immune response in the brain of autistic patients. J NEUROIMMUNOL. 2009;207(1–2):111–6.
30. Hylén U, McGlinchey A, Orešič M, Bejerot S, Humble MB, Särndahl E, Hyötyläinen T, Eklund D. Potential transdiagnostic lipid mediators of inflammatory activity in individuals with serious mental illness. FRONT PSYCHIATRY. 2021;12:778325.
31. Siniscalco D, Schultz S, Brigida AL, Antonucci N. Inflammation and Neuro-Immune dysregulations in autism spectrum disorders. PHARMACEUTICALS-BASE 2018, 11(2): 56.
32. Ferencova N, Visnovcova Z, Ondrejka I, Hrtanek I, Bujnakova I, Kovacova V, Macejova A, Tonhajzerova I. Peripheral inflammatory markers in autism spectrum disorder and attention deficit/hyperactivity disorder at adolescent age. INT J MOL SCI 2023, 24(14) : 11710.
33. Capiotto F, Romano CG, Mirlisenna I, Mazza A, Cicinelli G, Lauritano C, Keller R. Dal Monte O: autonomic and hedonic response to affective touch in autism spectrum disorder. AUTISM RES. 2024;17(5):923–33.
34. Cascio CJ, Foss-Feig JH, Heacock J, Schauder KB, Loring WA, Rogers BP, Pryweller JR, Newsom CR, Cockhren J, Cao A, et al. Affective neural response to restricted interests in autism spectrum disorders. J CHILD PSYCHOL PSYC. 2014;55(2):162–71.
35. Mostafa GA, Bjørklund G, Urbina MA, Al-Ayadhi LY. The levels of blood mercury and inflammatory-related neuropeptides in the serum are correlated in children with autism spectrum disorder. METAB BRAIN DIS. 2016;31(3):593–9.
36. Harkins CM, Handen BL, Mazurek MO. The impact of the comorbidity of ASD and ADHD on social impairment. J AUTISM DEV DISORD. 2022;52(6):2512–22.
37. Wu YT, Tsao CH, Huang HC, Yang TA, Li YJ. Relationship between motor skills and Language abilities in children with autism spectrum disorder. PHYS THERAPY . 2021, 101(5) :pzab033.
38. Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4 + and CD8 + T cells in autism. J NEUROIMMUNOL. 1998;85(1):106–9.
39. Singh S, Yazdani U, Gadad B, Zaman S, Hynan LS, Roatch N, Schutte C, Marti CN, Hewitson L, German DC. Serum thyroid-stimulating hormone and interleukin-8 levels in boys with autism spectrum disorder. J NEUROINFLAMM. 2017;14(1):113.
40. Shen Y, Li Y, Shi L, Liu M, Wu R, Xia K, Zhang F, Ou J, Zhao J. Autism spectrum disorder and severe social impairment associated with elevated plasma interleukin-8. PEDIATR RES. 2021;89(3):591–7.
41. Lee GR. The balance of Th17 versus Treg cells in autoimmunity. INT J MOL SCI . 2018, 19(3) :730.
42. Hughes HK, R JM, Ashwood P. Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). BRAIN BEHAV IMMUN. 2023;108:245–54.
43. Yousefi B, Kokhaei P, Mehranfar F, Bahar A, Abdolshahi A, Emadi A, Eslami M. The role of the host Microbiome in autism and neurodegenerative disorders and effect of epigenetic procedures in the brain functions. NEUROSCI BIOBEHAV R. 2022;132:998–1009.

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