

Maternal immune activation imprints postnatal Treg insufficiency driving meningeal neuroinflammation and reversible autistic behaviors in mice and humans

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Abstract

Maternal immune activation (MIA) during pregnancy induces autism spectrum disorder (ASD)-like phenotypes in offspring. While maternal IL-17 signaling has been implicated in prenatal brain alterations, the postnatal mechanisms sustaining this phenotype remain unknown. Here, we show that MIA in mice imprints a persistent postnatal insufficiency of regulatory T cells (Tregs) in offspring, characterized by altered DNA methylation, transcriptional dysregulation, and impaired *in vivo* suppressive function. This Treg insufficiency drives sustained meningeal inflammation marked by infiltration of neutrophils and IL-17-producing immune cells, resulting in region-specific alterations in brain structure and functional connectivity. Functional MRI revealed hypoconnectivity in sensory networks together with hyperconnectivity in regions involved in emotional regulation, reward processing, and interhemispheric cognitive and motor integration.

Postnatal stimulation of endogenous Tregs with low-dose interleukin-2 (IL-2) in MIA offspring expanded meningeal Tregs, eliminated IL-17-producing cells, resolved meningeal inflammation, normalized brain connectivity, and reversed MIA-induced ASD-like behaviors in adulthood.

Extending these findings to humans, two children with ASD associated with documented maternal immune activation received treatment with low-dose IL-2 for six months. Treatment expanded circulating Tregs and was associated with marked and progressive clinical improvement across all assessed domains - social communication, socialization, interpersonal relationships, adaptive coping, and play. To our knowledge, this represents an unprecedented pharmacological improvement of core ASD symptoms.

Together, our results redefine MIA-ASD as a chronic neuroimmune disorder amenable to therapeutic intervention, and establish Treg stimulation as a clinically actionable strategy with implications extending beyond autism to other MIA-associated neuropsychiatric disorders.

Introduction

Autism spectrum disorder (ASD) affects approximately 1 in 36 children and remains without effective pharmacological treatment targeting core symptoms. Among the environmental factors robustly associated with increased ASD risk¹, maternal immune activation (MIA) triggered by infections or autoimmune flares during pregnancy stands out for the strength of both epidemiological and experimental evidence². Historical and contemporary epidemiological evidence supports this link: children born during the 1918–1919 influenza pandemic showed increased rates of schizophrenia and ASD^{3,4}; rubella infection during pregnancy conferred up to a 10% risk of ASD in exposed children^{5,6}; and emerging data suggest that gestational COVID-19 infection may elevate ASD risk⁷. Likewise, maternal autoimmune diseases are associated with a twofold increase in the likelihood of ASD in offspring⁸.

Animal models establish a causal relationship between MIA and ASD. In mice, administration of polyinosinic:polycytidylic acid [poly(I:C)] during mid-gestation induces a robust MIA response that leads

to core ASD-like phenotypes in the offspring, including deficits in social interaction, communication, and increased repetitive behaviors⁹. Mechanistic studies have identified maternal T helper 17 (Th17) cells and their secretion of the pro-inflammatory cytokine interleukin-17a (IL-17a) as critical mediators of this phenotype. Genetic and pharmacologic blockade of IL-17a during gestation prevents the development of ASD-like behaviors in offspring, establishing maternal IL-17a as necessary for MIA-induced ASD (MIA-ASD)¹⁰. The current paradigm is that IL-17a crosses the placenta and binds to receptors on fetal neurons, where it alters cortical development and synaptic organization^{10–12}. However, this prenatal IL-17a-dependent mechanism may not fully account for the persistence of ASD-like features into adulthood, raising the question of whether postnatal immune mechanisms actively sustain the pathological state and whether targeting them could reverse established disease.

Th17 cells are tightly regulated by regulatory T cells (Tregs), a specialized subset of CD4⁺ T cells essential for immune homeostasis, suppression of inflammation, and maintenance of peripheral tolerance¹³. Beyond these classical immune-regulatory functions, Tregs also promote tissue repair and regeneration through the production of factors such as amphiregulin¹⁴, limit neuroinflammation¹⁵, suppress astrogliosis¹⁴, promote oligodendrocyte differentiation and remyelination¹⁶, and modulate microglial polarization toward neuroprotective states¹⁵.

A decrease in splenic and mesenteric Tregs has been described in MIA offspring¹⁷. Similarly, we and others previously reported a marked reduction in circulating Treg numbers and a disrupted Treg/Th17 balance in individuals with ASD¹⁸. These findings suggest that Treg insufficiency may contribute to the postnatal perpetuation of ASD pathology. Here, using the well-established poly(I:C) model of MIA-ASD, we test this hypothesis and show that Treg insufficiency is a central and persistent feature of the condition, driving meningeal inflammation and structural and functional brain abnormalities that are reversible by Treg-targeted immunotherapy with low-dose IL-2 (IL-2_{LD}). Building on these preclinical findings, and given the well-established safety profile of IL-2_{LD} as a Treg-selective immunotherapy in multiple autoimmune conditions including in children, we extended this approach to two children with ASD associated with documented maternal immune activation.

Results

Maternal immune activation imprints Treg insufficiency in offspring.

Pregnant mouse dams were injected at embryonic day 12.5 (E12.5) with either poly(I:C) to induce MIA or PBS as a control. Offspring from poly(I:C)-treated dams (MIA_{F1}) mice exhibited a significant reduction in the frequency of blood Tregs compared to control offspring (Ctrl_{F1}) (**Fig.1A**). The Treg numerical insufficiency was systemic, also observed in secondary lymphoid organs (**Fig.1B**) and the meninges (**Fig.1C**) in which Tregs are readily detected (**Supplementary Fig.1**)¹⁹. Concomitant with this Treg

reduction, MIA_{F1} mice showed an increased proportion of Th17 cells (**Fig.1D**), resulting in a markedly decreased Treg/Th17 ratio (**Fig.1E**), mirroring the imbalance previously reported in ASD patients¹⁸.

To further investigate how MIA affects Treg in offspring, we compared bulk RNA sequencing (RNA-seq) of Tregs from MIA_{F1} and Ctrl_{F1}. This analysis identified 62 differentially expressed genes (DEGs) (**Fig.1F**). Principal component analysis (PCA) based on these DEGs separated the MIA_{F1} and Ctrl_{F1}, confirming the biological relevance of these transcriptomic changes (**Fig.1G**). Notably, the first principal component was driven primarily by two genes. The downregulated *Vdr* gene (encoding the vitamin D receptor) is a critical regulator of Treg differentiation²⁰, and genetic variants in *Vdr* have been linked to ASD²¹. The reduced expression of *Vdr* in MIA_{F1} Tregs may therefore contribute mechanistically to their deficiency. The upregulated *Bcas1* (encoding the brain-enriched myelin-associated protein 1) is a gene expressed in immature oligodendrocytes²² and previously identified as an ASD candidate gene^{23,24}, that may reflect the influence of an inflamed or developmentally altered brain environment. Consistent with this transcriptional reprogramming, gene ontology (GO) analysis further indicated that MIA_{F1} Tregs were enriched for “cellular components” (**Fig.1H**) and “biological process” (**Fig.1I**) GO terms associated with brain development and function. Given the known property of Tregs to adopt a transcriptomic profile influenced by their tissue environment²⁵, this underscores that MIA imprints Tregs with a program converging on neuroimmune interaction.

Given that MIA is known to induce epigenetic changes in fetal T cells^{26,27}, we also examined the DNA methylation landscape of MIA_{F1} Tregs. A genome-wide methylome analysis of Tregs revealed 52 differentially methylated regions (DMRs) between MIA_{F1} and Ctrl_{F1} Tregs (**Supplementary Fig.2A**). Unsupervised clustering of these DMRs identified two distinct modules that effectively distinguished MIA_{F1} from Ctrl_{F1} samples (**Supplementary Fig.2B**). Principal component analysis further demonstrated a clear separation between MIA_{F1} and Ctrl_{F1} samples (**Supplementary Fig.2C**), underscoring the functional relevance of these epigenetic alterations. The Treg-specific demethylated region (TSDR) within the *FoxP3* locus remained unaffected, indicating that the lineage integrity of Tregs was preserved²⁸. Taken together, these findings suggest that MIA induces an epigenetically imprinted functional impairment of Tregs in offspring.

Given these Treg abnormalities, we next evaluated whether MIA also impairs Treg function. In a standard *in vitro* suppression assay, Tregs from MIA_{F1} offspring did not show a defect in their ability to suppress effector T cell proliferation, compared to Ctrl_{F1} Tregs (**Fig.1J**). However, this assay predominantly measures the capacity of Tregs to deprive effector T cells of IL-2 and does not fully capture the breadth of Treg functional activity. To uncover a subtler Treg insufficiency, we aimed to broadly assess Treg performance *in vivo*. We induced MIA in pregnant non-obese diabetic (NOD) mice. A fraction of NOD mice develop a spontaneous diabetes that is largely due to a Treg insufficiency that licenses the autoimmune destruction of pancreatic β cells²⁹. Of note, the incidence of NOD mice diabetes is exacerbated by Treg depletion and prevented by Treg stimulation with IL-2³⁰. We thus reasoned that if

MIA induces a subtle Treg insufficiency, this should add up to the intrinsic Treg insufficiency of the NOD background to exacerbate diabetes, as Treg depletion does. NOD MIA_{F1} offspring indeed developed diabetes more rapidly and with higher incidence than NOD Ctrl_{F1} (**Fig.1K**), providing an *in vivo* readout of an impaired Treg fitness.

Maternal immune activation induces a severe inflammatory meningeal environment in offspring

Given the systemic Treg deficiency and the transcriptional and epigenetic changes observed in MIA_{F1} offspring, we next examined the impact of MIA on the brain immune environment, with a particular focus on the meninges. Indeed, meningeal immune cells have emerged as key regulators of brain development, homeostasis, and cognitive function^{31–33} and we had observed reduced numbers of mTregs in MIA_{F1} offspring. To further explore the meningeal immune environment, we analyzed CD45⁺ meningeal cells using single-cell RNA sequencing (sc-RNAseq) and flow cytometry.

UMAP analysis of quality-controlled sc-RNAseq data (**Supplementary Fig.3**) revealed distinct clusters corresponding to B cells (orange), T cells (light blue), macrophages (green), monocytes (dark blue), dendritic cells (violet), and neutrophils (red) (**Fig.2A**). Compared to Ctrl_{F1} (grey), MIA_{F1} (blue) meninges exhibited an increase in monocytes, neutrophils, and B cells, each displaying altered transcriptional profiles, with mostly upregulated genes in monocytes, neutrophils and B cells, and downregulated ones in T cells (**Fig.2B**). These changes suggest an ongoing local inflammation driven by IL-17a, which is known to promote monocyte and neutrophil recruitment in inflamed tissues³⁴. In line with these observations, gene ontology functional enrichment analyses of the transcriptome of total meningeal CD45⁺ cells found upregulation of pro-inflammatory pathways, production of reactive oxygen species and increased apoptotic signaling (**Fig.2C**).

Using sc-RNAseq, meningeal Tregs (mTregs) could be identified by Foxp3 expression within the T cell cluster (**Fig.2D**). MIA_{F1} mice exhibited a markedly and significantly reduced proportion of mTregs compared to Ctrl_{F1} (**Fig.2E**). Despite this reduction, MIA_{F1} mTregs showed increased expression of CD69, a marker of recent activation and tissue residency, suggesting their engagement in response to local inflammation (**Fig.2F**). Furthermore, a subset of these mTregs expressed *Areg* (**Fig.2F**), which has been shown to suppress neurotoxic astrogliosis during neuroinflammation¹⁴, as well as *Spp1* (also known as Osteopontin) (**Fig.2F**), a molecule that enhances microglial reparative function, promotes oligodendrocyte differentiation, and supports white matter repair³⁵. These findings suggest that, although meningeal Tregs are numerically deficient in MIA_{F1}, the remaining cells adopt a compensatory neuroprotective phenotype in an attempt to control local inflammation and support tissue repair.

To further characterize the meningeal T cell compartment in MIA_{F1} mice, we performed detailed flow cytometry analysis using unsupervised clustering to avoid bias in cell population gating. In Ctrl_{F1} mice,

most meningeal T cells are ROR γ t⁺ IL-17A-producing T cells (violet), accompanied by smaller populations of $\gamma\delta$ T cells (yellow) and Tregs (red and green) (**Fig.2G**) Notably, about 80% of the meningeal Tregs in control mice expressed ROR γ t, identifying them as Th17-like Tregs (green), a Treg subset previously described in barrier tissues^{36,37} and known to play context-dependent roles in immune regulation³⁸.

Strikingly, in MIA_{F1} mice, we observed a dramatic expansion of IL-17A-producing T cells (**Fig.2H**), which made up over 75% of the meningeal T cell compartment (**Fig.2H**), reflecting a highly pro-inflammatory environment. In parallel, the population of classical Tregs (ROR γ t⁻) was reduced by more than 2-fold, while the proportion of Th17-like Tregs (ROR γ t⁺ Foxp3⁺) was increased by 5-fold (**Fig.2H**).

Meningeal inflammation disrupts brain structure and connectivity in MIA offspring

To evaluate the functional consequences of meningeal inflammation, we performed high-resolution brain imaging using an 11.7 Tesla MRI, allowing for detailed structural and functional analysis. We assessed both brain anatomy and resting-state functional connectivity (rsFC).

Anatomical MRI first revealed that MIA_{F1} mice exhibited significantly increased volume dispersion in ten brain regions compared to Ctrl_{F1} (**Fig.3A and Supplementary Fig.4**), particularly in areas implicated in social behavior abnormalities in ASD³⁹. These included the insular cortex (agranular insular and gustatory areas) (**Fig.3A**), basal ganglia (striatum, amygdala, hypothalamus, and claustrum), and anterior cingulate cortex (**Supplementary Fig.4**). Noteworthy, although poorly understood, an increased regional volume dispersion has also been reported in humans with ASD^{66,67}.

In addition to volume dispersion, two brain regions showed a significant variation in their volumes. The ectorhinal cortex, a key sensory-associative area, and the perirhinal cortex, a polymodal associative hub linking the cortex and hippocampus both had a significant increase in their volumes (**Fig.3B,C**). These two adjacent regions of the medial temporal lobe have previously been associated with altered myelination in MIA^{ASD} mouse models⁴⁰, and humans with ASD show enlarged temporal lobes⁴¹ whose structural changes have been linked to social and communication impairments⁴². Importantly, both rare and common ASD-associated gene variants show enriched expression in the temporal lobe⁴³, further underscoring the relevance of these structural abnormalities.

As functional MRI better predicts ASD-related symptoms than anatomical MRI⁴⁴, we next analyzed rsFC in Ctrl_{F1} and MIA_{F1} mice. MIA_{F1} exhibited widespread rsFC alterations, including both hypo- and hyper-connectivity patterns (**Fig.3D**). Notably, and consistent with our anatomical findings, we observed hypoconnectivity between the adjacent perirhinal and ectorhinal cortices. Additional hypoconnected regions primarily involved long-range sensory circuits: connections between the primary sensory cortex

and gustatory areas, the orbital cortex, olfactory regions, and higher-order sensory integration hubs such as the subplate cortex⁴⁵ and the claustrum⁴⁶. Interestingly, both the olfactory cortex and subplate region are considered hotspots of aberrant connectivity in multiple ASD models^{47–49}.

Consistent with these observations and previous reports⁹, MIA_{F1} offspring displayed a robust ASD-like phenotype when assessed using classical behavioral assays. These included: (i) early communication deficits, measured by reduced maternal isolation-induced ultrasonic vocalizations (USVs) number (**Fig.3E**), duration (**Fig.3F**) and modulation (**Fig.3G**); (ii) increased repetitive and stereotypic behaviors in the marble burying test⁵⁰ (**Fig.3H**); and (iii) diminished social interactions in the three-chamber social approach test (**Fig.3I**), as compared to control offspring (Ctrl_{F1}). To more accurately assess social behavior in an ecologically valid setting, we additionally employed the Live Mouse Tracker (LMT) system, a fully automated, continuous monitoring platform that identifies and quantifies social interactions among freely moving mice over a 14-hour period⁵¹. Applying this approach for the first time in the context of MIA-induced ASD, we found that adult MIA_{F1} mice exhibited fewer social contacts of all types (side-to-side, opposite side-to-side, oral-oral, oral-genital), formed smaller social groups (pairs or trios), and spent more time moving alone (**Fig.3J**). These findings provide robust evidence that MIA induces long-lasting ASD-like behavioral abnormalities in offspring. Together, these data indicate that meningeal inflammation drives the structural and functional brain alterations underlying MIA-induced ASD-like behaviors.

The inflammatory meningeal environment in MIA offspring is reversible by Treg stimulation

Our findings reveal a pronounced meningeal inflammation in MIA_{F1} mice, characterized by a significant increase in IL-17–producing cells and neutrophils alongside marked Treg abnormalities. An important question is whether these Treg abnormalities are a cause or a consequence of the meningeal inflammation. Indeed, Tregs control inflammation, but are also destabilized by inflammation⁵². To investigate this “chicken-or-egg” dilemma, we investigated whether boosting Tregs could alleviate the meningeal inflammation. To this end, we treated mice with low-dose IL-2 (IL-2_{LD}), a strategy known to selectively expand and activate Tregs both systemically and within tissues⁵³, including the brain¹⁵. To ensure sustained IL-2 delivery without the stress from repeated injections, we administered a single dose of an adeno-associated virus encoding IL-2 (AAV_{IL2}) that allows long-term in vivo stimulation of Tregs⁵³, or a control empty AAV (AAV_E). The different groups were identified as Ctrl_{F1} and MIA_{F1} if they received the AAV_E; Ctrl_{F1}-IL2 and MIA_{F1}-IL2 if they received AAV_{IL2}.

IL-2 treatment was initiated at post-natal week 3, a time point at which offspring already exhibit ASD-like behaviors, as evidenced by abnormal ultrasonic vocalizations at post-natal day 7. IL-2_{LD} treatment resulted in a significant expansion of Tregs in the blood (**Fig.4A**) which resulted in normalization of the Treg/Th17 ratio (**Fig.4B**). This marked Treg activation had profound effects on the meningeal inflammation (**Fig.4C**). The dramatic infiltration by IL-17 producing cells (Violet) in MIA_{F1} was dramatically reduced in MIA_{F1}-IL2(>5fold) to levels even lower than in control mice (**Fig.4D**).

Concomitantly, in MIA_{F1}-IL2, the IL-17 producing Tregs (green) were also reduced by >5fold while the classic meningeal Tregs (red) were expanded by >5fold (**Fig.4C and Fig. 4D**). In MIA_{F1}-IL2 mice, IL-17-producing cells were almost eliminated, classical Tregs were restored, and the previously expanded Th17-like Treg population was no longer detectable. Accordingly, the nearly 50-fold increase in the Treg Th17-like/Treg ratio in MIA_{F1} compared with Ctrl_{F1} was fully normalized in mice receiving IL-2_{LD} (**Fig.4D**).

Together, these results demonstrate that the meningeal immune landscape in MIA^{ASD} is marked by a chronic pro-inflammatory state driven by excess IL-17-producing cells and neutrophils and a paucity of Tregs, which is almost fully reversed by boosting Tregs via IL-2 treatment. This establishes a causal link between Treg deficiency and neuroinflammation in MIA^{ASD}.

Treg stimulation with low dose IL-2 reverses brain structure and connectivity abnormalities in MIA offspring.

We next assessed the effect of the Treg stimulation and its associated reduction in meningeal inflammation on the brain structure abnormalities of MIA_{F1}. Most volume dispersion abnormalities were improved in MIA_{F1}-IL2 mice, although not fully normalized (**supplementary Fig.4B**). The increase in the ectorhinal and perirhinal volumes was normalized in MIA_{F1}-IL2 (**Fig.4E,F**). Thus, MIA-induced neuroanatomical alterations were largely improved by Treg-based immunomodulation.

Likewise, IL2_{LD} largely restored connectivity in MIA_{F1}-IL2 mice (**Fig.4G**). All hypo-connected circuits were normalized, except for a continued hypoconnectivity between the claustrum and cerebellar cortex. IL-2_{LD} treatment also normalized the hyperconnectivity patterns, except for a persistent hyperconnectivity between the medulla and extrapyramidal motor tracts.

Treg stimulation with low dose IL-2 reverses core ASD-like behaviors in MIA offspring

The resolution of the meningeal inflammation and its associated improvements in brain structure and connectivity led to major improvements in ASD like behaviors in MIA_{F1}-IL2 mice. Although AAV_{IL2} has no effect on the behavior of Ctrl_{F1} (**Supplementary Fig.5**), repetitive behaviors were fully rescued in MIA_{F1}-IL2 mice (**Fig.4H**). IL-2_{LD} treatment significantly enhanced social interaction metrics (**Fig.4I-J**) such as number of contacts, quality of social configurations, and frequency of social approaches in MIA_{F1} mice. Together, these results demonstrate that IL-2-mediated stimulation of Tregs significantly reverses core autistic-like behaviors in MIA_{F1} offspring, underscoring a causal link between Treg dysfunction and ASD-like phenotypes.

Treg stimulation with low dose IL-2 improves core behaviors in children with autism

Given these results, and our experience in treating patients with IL-2^{54,55}, including children from 6 year of age⁵⁶, we treated two children with ASD under the frame of the EXPECT protocol (NCT04923854) conducted in the Excellence Centre for Autism & Neuro-developmental Disorders (InovAND-Robert Debré Hospital, Paris, France), and approved by the local ethics committee (2021–27, N° IDRCB: 2021-A00489-32). The two patients were included in this case series after their legal guardians had received detailed information regarding the potential risks and benefits and provided their written informed consent.

Patients received subcutaneous IL-2 at a dose of 0.8 MIU/m² for 5 consecutive days every 3 weeks. Clinical evaluations were performed at baseline, 3 months, and 6 months. Behavioral and developmental assessments were performed using state-of-the-art methods that included (i) the Social Responsiveness Scale, Second Edition (SRS-2), a caregiver-completed questionnaire measuring the severity of social impairment; (ii) the Autism Diagnostic Observation Schedule (ADOS-2), a standardized semi-structured observational assessment of social communication and restricted or repetitive behaviors, administered by trained neuropsychologists specialized in autism spectrum disorder; and (iii) the Vineland Adaptive Behavior Scales, Second Edition (VABS-2), a caregiver interview assessing adaptive functioning in the domains of communication, socialization, and daily living skills.

Patient 1 (in blue) is a 12-year-and-4-month-old boy. Prenatal history was notable for maternal toxoplasmosis during the second trimester. Early motor development was largely preserved, with sitting at 5–6 months, standing at 10 months, and independent walking at 11 months. In contrast, social communication and language were markedly delayed, with absent babbling during the first year of life, delayed first words, and delayed proto-imperative pointing, which emerged after 2 years of age. ASD was diagnosed at around 2.5 years of age and later confirmed by standardized assessments. After 5 days of IL-2_{LD}, Tregs percentages increased, with parallel rises in the proportions of cells with activation markers such as CD39+, GITR+, ICOS+, CD73+, CTLA4+ and CD25 MFI; in contrast, there was a decrease in LAG3+ Tregs (**Fig5.A**). Clinical evolution showed progressive clinical improvements over time. SRS-2 score improved from the severe range at M0 to the moderate range at M2 and M6 (**Fig5.B and supplementary Table 1**). His ADOS-2 total score decreased from the autism range at M0 to the ASD range at M2 and M6, with his ADOS-2 calibrated severity score improved from the moderate range at M0 to the mild range M6 (**Fig.5C,D and supplementary Table 1**). Having characterized symptom changes with the SRS-2 and ADOS-2 scales, we next evaluated whether these shifts translated into measurable effects on real-world adaptive functioning using the VABS-2 scale. Regarding socio-adaptive behaviors, VABS-2 communication scores improved progressively over time, with increases in both total scores (**Fig.5E left**) and in the expressive and receptive subscores (**Fig.5E right**); as expected, the written subscore remained stable. There was a similar evolution for the VABS-2 socialization scores, with increases in both total scores (**Fig.5F left**) and in the coping, interpersonal relationship and play subscores (**Fig.5F right**).

Patient 2 (in red) is an 8-year-and-1-month-old boy. Prenatal and perinatal history was notable for maternal systemic lupus erythematosus, with several systemic flares during pregnancy and fetal

atrioventricular block detected at 5 months' gestation. He required neonatal hospitalization for his lupus-related atrioventricular block and underwent pacemaker implantation at 18 months of age. Early motor development was normal, with first steps at around 12 months, whereas language and social communication were more affected, with first words between 12 and 18 months followed by regression after 18 months. He currently does not use complex sentences and communicates with PECS. Autism spectrum disorder with intellectual disability was confirmed. After 5 days of subcutaneous IL-2_{LD}, circulating Tregs expanded with a phenotypic signature consistent with patient 1, including increases in CD39⁺, GITR⁺, ICOS⁺, CD73⁺, and CTLA4⁺ Treg subsets and CD25 MFI, and a concomitant decrease in LAG3⁺ Tregs (**Fig. 5A**). Clinical evolution showed similar improvements over time. SRS-2 score improved from the moderate range at M0 to the mild range at M2 and the within-normal range at M6 (**Fig. 5B and supplementary Table 1**). His ADOS-2 total score similarly decreased from the autism range at M0 to the ASD range at M6, with an ADOS-2 calibrated severity score improvement from the moderate range at M0 to the mild range at M2 and M6 (**Fig. 5C,D and supplementary Table 1**). Regarding socio-adaptive behaviors, VABS-2 communication scores and subscores improved progressively over time (**Fig. 5E left and right**) while the written subscore remain stable. There was a similar evolution for the VABS-2 socialization scores, with remarkable increases in both total scores (**Fig. 5F left**) and in the coping, interpersonal relationship and play subscores (**Fig. 5E left**).

In both patients, clinical improvements were progressive, spanning the full duration of treatment, and were accompanied by qualitative gains in spontaneous pro-social behaviors and the emergence of short phrases, changes of clear functional significance. No serious treatment-related adverse events were observed.

Discussion

Maternal immune activation imprints a long-lasting insufficiency of Tregs in _{MIA}ASD offspring, which sustains chronic meningeal inflammation, drives structural and functional brain abnormalities, and culminates in core ASD-like behaviors. Indeed, therapeutic stimulation of Tregs fully reverses these abnormalities, underscoring their causal role and pointing to a new conceptual and therapeutic framework for autism. While previous work has identified maternal Th17 responses and IL-17a as key mediators of fetal brain abnormalities in MIA-induced ASD models, our findings reveal that a previously unrecognized postnatal Treg insufficiency is a critical driver of chronic pathology. IL-17a exposure during gestation may initiate neurodevelopmental disruption, but our data show that persistent Treg insufficiency in offspring sustains inflammation well into adulthood. This reframes ASD not merely as the result of a prenatal insult, but as a chronic, immune-mediated brain disorder amenable to intervention.

A previous study reported a quantitative deficiency of splenic and mesenteric Tregs in MIA_{F1}¹⁷. Here, we report a quantitative meningeal Treg deficiency, associated with conversion of a substantial fraction of meningeal Tregs into RORγt⁺Tregs. Functionally, Tregs in MIA_{F1} have a limited deficit that could only be

unmasked in NOD mice in which the increased diabetes incidence following MIA in NOD dams provides an *in vivo* readout of a subtle impaired Treg fitness. Of note, a directly causal relationship between Tregs and ASD cannot be assessed with the classical models of Treg ablation, as the rapid catastrophic inflammation and multiorgan autoimmunity induced by it would confound the interpretation of results.

Methylomic and transcriptomic analyses offer initial mechanistic insights into the Treg insufficiency: MIA does not destabilize Treg identity (Foxp3 TSDR remains intact) but alters critical functional pathways. In particular, the vitamin D receptor gene (*Vdr*) is markedly downregulated in MIA_{F1} Tregs. As *Vdr* is essential for Treg differentiation and function²⁰, this downregulation may contribute to both quantitative and qualitative defects. Supporting this, genetic variants in *Vdr* have been associated with ASD²¹, and vitamin D deficiency is associated with increased risk of Treg-dependent autoimmune diseases⁵⁷. These findings identify *Vdr* as a candidate mediator and therapeutic target in MIA_{F1} ASD, warranting evaluation in future studies.

The meningeal compartment is particularly affected by Treg insufficiency, exhibiting a profound inflammatory shift. Neutrophils and IL-17–producing T cells accumulate in the meninges of MIA_{F1} mice, and this inflammation is fully resolved upon Treg stimulation. mTregs in MIA_{F1} express high levels of amphiregulin (Areg) and osteopontin (Spp1), molecules each implicated in neuroprotection and repair. Areg suppresses neurotoxic astrogliosis¹⁴, while Spp1 promotes microglial repair and oligodendrocyte maturation¹⁶. Thus, Tregs may engage in compensatory repair programs in response to inflammation.

We also identify an abundant population of Th17-like Tregs in the meninges - unreported in this context - whose expansion in MIA_{F1} may reflect adaptation to an IL-17-rich environment. These cells can play dual roles: Th17-like Tregs have been shown to be reparative or pro-inflammatory depending on context, such as cancer⁵⁸ or brain autoimmunity^{38,59}. These cells disappear following IL-2 treatment, which also resolves brain inflammation and restores brain connectivity. This supports a pathological role and highlights the central role of Tregs in shaping the meningeal immune landscape.

The brain–immune interface has emerged as an important area of investigation in neuropsychiatric diseases^{60–62}. Our findings place the meninges and their immune constituents at the center of ASD pathophysiology. The expansion of IL-17-producing T cells and neutrophils, along with Treg insufficiency and phenotypic plasticity, provides a mechanistic bridge between peripheral immune dysfunction and central nervous system (CNS) abnormalities. For the first time, we assessed ASD-relevant brain structure and connectivity in the MIA_{F1} model using high-field anatomical and functional MRI. Structural imaging revealed increased volume dispersion in multiple brain regions implicated in social behavior - including the insular cortex, anterior cingulate cortex, basal ganglia, and temporal lobe - closely paralleling findings in individuals with ASD³⁹. The pathophysiological basis of volume dispersion, also reported in humans^{63,64}, remains unclear but may reflect instability or dynamic dysregulation within these affected regions. That these abnormalities are reversed by IL-2 therapy supports their inflammatory origin.

Resting-state fMRI revealed alteration in functional connectivity in MIA_{F1} mice. Hypoconnected regions included long-range sensory pathways and associative hubs such as the subplate and claustrum, previously implicated in ASD⁴⁶. Such hypoconnectivity may contribute to the reduced social communication observed in ASD. Hyperconnectivity was noted between the medial forebrain bundle and the amygdala, which are key components of the emotional regulation and reward circuitry^{65–67} and have been implicated in the socio-emotional impairments seen in children with ASD³⁹. Also, hyperconnectivity between the striatum and the corpus callosum, regions critical for coordinating interhemispheric cognitive and motor functions, could be associated with the restricted and repetitive behaviors characteristic of ASD^{68–70}. That Treg stimulation resolves meningeal inflammation and normalizes brain structure and connectivity further underscores the central role of Tregs in immune-brain communication.

The behavioral rescue observed following IL-2–mediated Treg stimulation provides direct evidence that core ASD-like features are reversible. This challenges the prevailing view of ASD as a fixed neurodevelopmental disorder and supports the conclusion that chronic inflammation is required to maintain the behavioral phenotype. While early neurodevelopmental insults may initiate the disease, our data show that ongoing immune dysfunction is required for its maintenance—and is therapeutically targetable.

Considering the known biology of Tregs and IL-2, the collective correction of Treg defects, meningeal inflammation, brain connectivity, and behavior by IL-2 strongly supports a central role for Tregs in ASD. Indeed, although IL-2 has pleiotropic effects that can affect B cells and NK cells, these cells do not have a clear role in ASD and the dominant effect of IL-2 at the dose used is by far on Tregs⁷¹. Of note, a previous study reported the beneficial effects of a Treg-based cell-therapy in a *Toxoplasma gondii*-induced MIA in mice. However, in this model, the therapeutic effect was only carried by Tregs (i) of maternal origin and more specifically by Tregs (ii) from mice with *Toxoplasma gondii*-induced MIA, i.e. pathogen specific⁷². This study did not test whether offspring Tregs could also have a therapeutic effect. A recent study investigated whether adoptive transfer of wildtype Tregs into MIA offspring could rescue immune dysregulation and behavioral abnormalities⁷³. This approach yielded modest reductions in peripheral Th17 cells, and behavioral rescue was limited to self-grooming in males and social novelty in females, with no improvement in core social approach behavior. Together, these cell-therapy studies underscore both the therapeutic relevance of Tregs in MIA-ASD and the superiority of endogenous Treg stimulation with IL-2 over adoptive transfer approaches. The latter are technically demanding, provide only transient Treg engraftment and yield incomplete behavioral rescue. By contrast, postnatal stimulation of endogenous Tregs achieves sustained Treg expansion, full resolution of meningeal inflammation, normalization of brain connectivity, and global behavioral rescue.

That postnatal offspring Treg stimulation with IL-2_{LD} is beneficial has substantial translational implications. IL-2_{LD} has shown efficacy in numerous preclinical models of neuroinflammation, including multiple sclerosis⁷⁴, Alzheimer disease⁷⁵, and now ASD^{74,75}. Clinically, IL-2_{LD} is under active investigation in autoimmune and neuroimmune conditions, and a recent trial reported improved survival

in ALS⁷⁶. Low dose IL-2 has demonstrated an excellent safety profile across indications⁷⁷. Based on these findings, which underscore IL-2_{LD} safety and potential in modulating CNS inflammation, we extended this approach to two children with _{MIA}ASD.

The clinical improvements observed in both patients deserve specific comment. First, regarding target engagement: both patients showed robust and consistent Treg expansion following low-dose IL-2, with phenotypic activation markers - including upregulation of CD39, GITR, ICOS, CD73, and CTLA4 and decreased LAG3 - mirroring the Treg signatures associated with therapeutic benefit in other IL-2 clinical programs^{55,71}.

Second, regarding the nature of the improvements: the gains observed span the core ASD domain of social communication and social interaction as captured by three independent validated instruments (SRS-2, ADOS-2, and VABS-2). This convergence across observer-completed, clinician-administered, and caregiver-interview tools, each capturing different facets of adaptive function, substantially reduces the likelihood of reporting bias and strengthens the validity of the clinical signal. Qualitatively, the emergence of short phrases and increased social engagement represent changes of clear functional significance.

Third, regarding timing: improvements were progressive and continued to accrue between months 3 and 6, suggesting that the clinical benefit reflects a sustained biological process rather than a transient pharmacological effect. This trajectory is consistent with the time course of Treg-mediated resolution of inflammation and subsequent neuroplastic remodeling and raises the possibility that longer treatment durations could yield further gains.

Fourth, current international recommendations support ASD-specific screening at 18 and 24 months, with diagnostic evaluation initiated as soon as parental concerns arise. We believe that although our results show improvements in children aged 8 and 12 years, earlier treatment would likely be even more beneficial. We have demonstrated safety of IL-2_{LD} in children down to 6 years old. We are now starting a phase 2a randomized placebo-controlled trial of low-dose IL-2 in children with ASD from this age (NCT07589842). The demonstration of safety in this setting should allow treating younger patients in the future.

Taken together, our findings establish a new framework of _{MIA}ASD as a chronic, immune-mediated disorder of the brain, sustained by postnatal Treg insufficiency and reversible through targeted immunotherapy. While IL-17a signaling during gestation may initiate neurodevelopmental disruption, it is the persistent postnatal Treg insufficiency that perpetuates pathology and that is therapeutically accessible. This paradigm shift repositions ASD not as a fixed neurodevelopmental lesion, but as a chronic neuroimmune condition amenable to intervention. Beyond autism, given that MIA has been implicated in schizophrenia and bipolar disorder, and that low-dose IL-2 has recently shown benefit in depression^{78,79}, the implications of this framework may extend broadly across neuropsychiatric disorders.

Declarations

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Author contributions:

Conceptualization: DK conceptualized the global project, with some specific contribution of PE and NT.

Investigation: Investigations were conducted by PE, GF, VM, NC, SB, AM, MM, SK, LM, GM, and TM

Visualization: Data visualization was performed by PE, UK, VM, TM, NT and DK

Formal analysis: NT performed the statistical analyses

Funding acquisition: DK

Validation: Results of experiments were validated by BY (epigenomics and transcriptomics), MR (immunology and clinical immunophenotyping), RD (child psychiatry and clinical assessment), DK (overall).

Supervision: DK carried out the overall supervision of the work.

Writing - original draft: PE, NT, DK

Writing - review & editing: all authors

Competing interests:

PE, MR, RD and DK are inventors of patents held by their institutions claiming the therapeutic use of IL-2.

List of Supplementary Materials

Data and materials availability:

All data, code, and materials used in the analysis are available upon request. Correspondence and requests for materials should be addressed to David Klatzmann.

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Figures

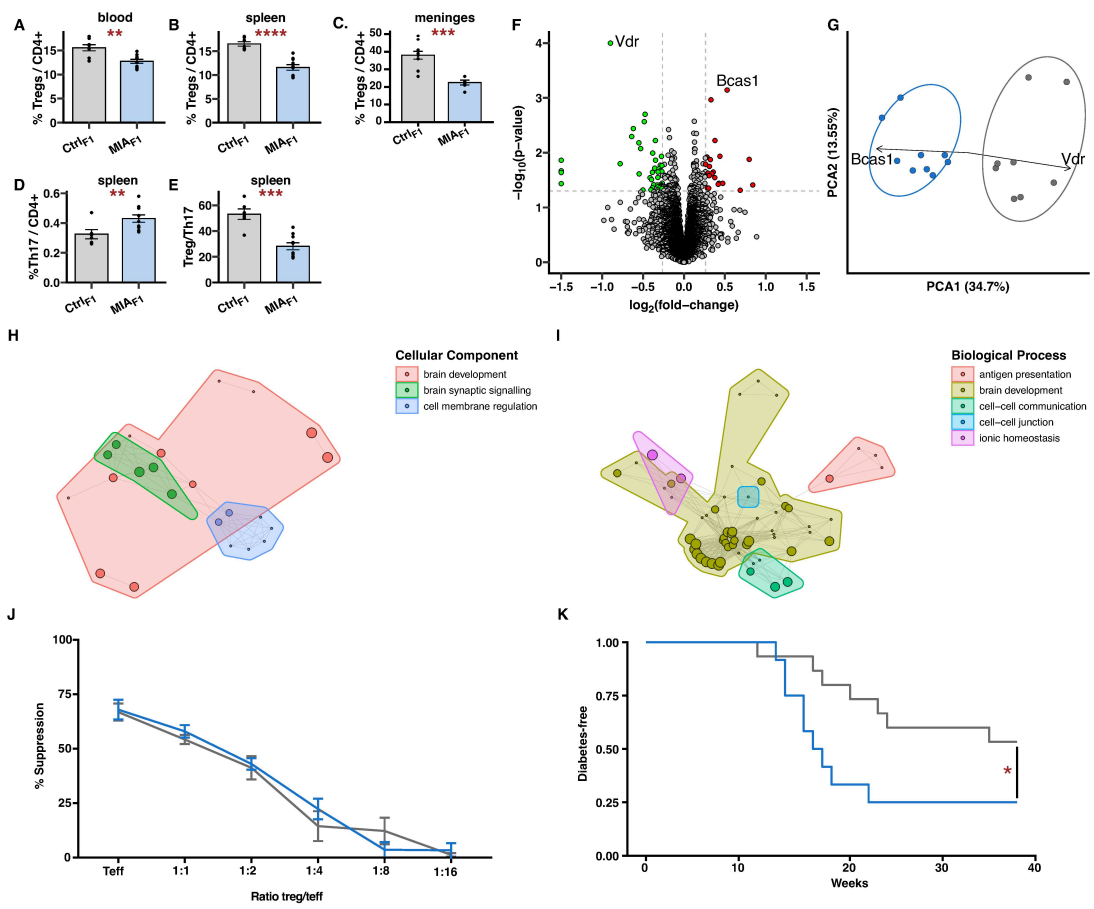


Figure 1

MIA induces long-term quantitative and functional abnormalities in offspring Tregs.

(A) Flow cytometry of blood Tregs from Ctrl_{F1} (n = 8, 3 experiments), MIA_{F1} (n = 9, 4 experiments). **(B)** Flow cytometry of spleen Tregs in Ctrl_{F1} (n = 7, 3 experiments) and MIA_{F1} (n = 10, 4 experiments). **(C)** Flow cytometry of meningeal Tregs of Ctrl_{F1} (n = 10, 3 experiments) and MIA_{F1} (n = 6, 3 experiments). **(D)**

Analysis of splenic Th17 cells of Ctrl_{F1} (n = 6, 3 experiments) and MIA_{F1} (n = 10, 4 experiments). **(E)** Treg/Th17 ratio in the spleen of Ctrl_{F1} (n = 6, 3 experiments), MIA_{F1} (n = 10, 4 experiments). **(F-G)** Bulk transcriptomic analysis of spleen Tregs. (F) volcano plot showing downregulated genes (yellow) and upregulated genes (red); (G) principal component analysis and correlation circle of differentially expressed genes. **(H-I)** Comparison of differentially expressed genes in MIA_{F1} vs. Ctrl_{F1} based on gene ontology for cellular functions (H) or biological functions (I). **(J)** In vitro suppressive assay using splenic Tregs from Ctrl_{F1} and MIA_{F1} mice (n = 3 mice per group, with three replicates for each group). **(K)** Proportion of diabetes-free offspring in Ctrl_{F1} vs. MIA_{F1} female NOD mice. Statistical analyses were performed using t-tests unless otherwise stated; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Results are expressed as mean ± SEM.

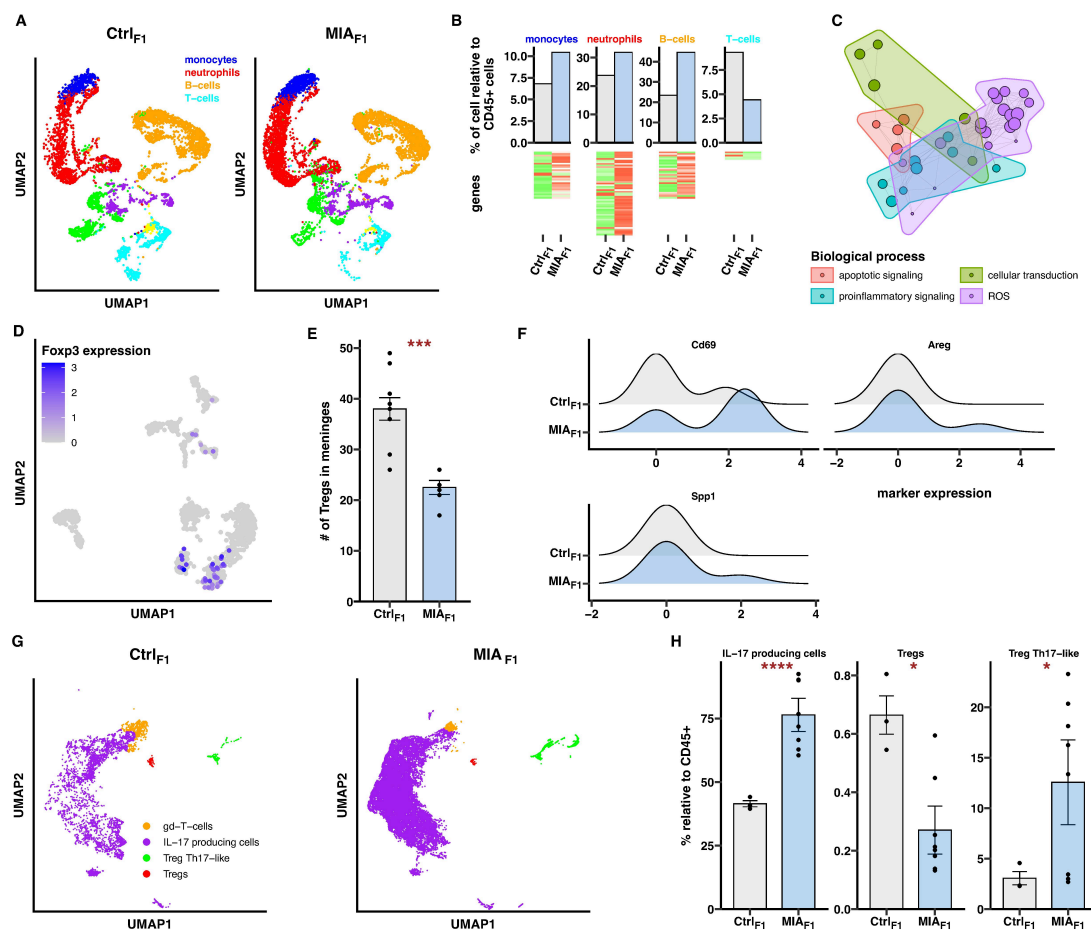


Figure 2

MIA induces a persistent IL-17a-driven meningeal inflammatory state.

(A) UMAP projection of single cell transcriptomics of CD45+ meningeal cells in Ctrl_{F1} (left) and MIA_{F1} (right). (B) Compositional analysis: percentage of monocytes, neutrophils, B cells and T cells relative to total CD45+ in all conditions with associated heatmap of differentially expressed genes. (C) Gene

ontology of upregulated pathways in MIA_{F1} and Ctrl_{F1} from sc-RNAseq data. (D) UMAP highlighting Foxp3+ cells among T lymphocytes from sc-RNAseq data. (E) Quantification of meningeal Treg populations from sc-RNAseq data. (F) Expression densities of CD69, AREG and SPP1 in Tregs (sc-RNAseq). (G) UMAPs of unsupervised flow cytometry from Ctrl_{F1} and MIA_{F1} adult mice (H) Cluster abundances based on UMAP analysis. Only males were used. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (t-tests, except for panel F: Kolmogorov Smirnov test). Data represent mean ± SEM.

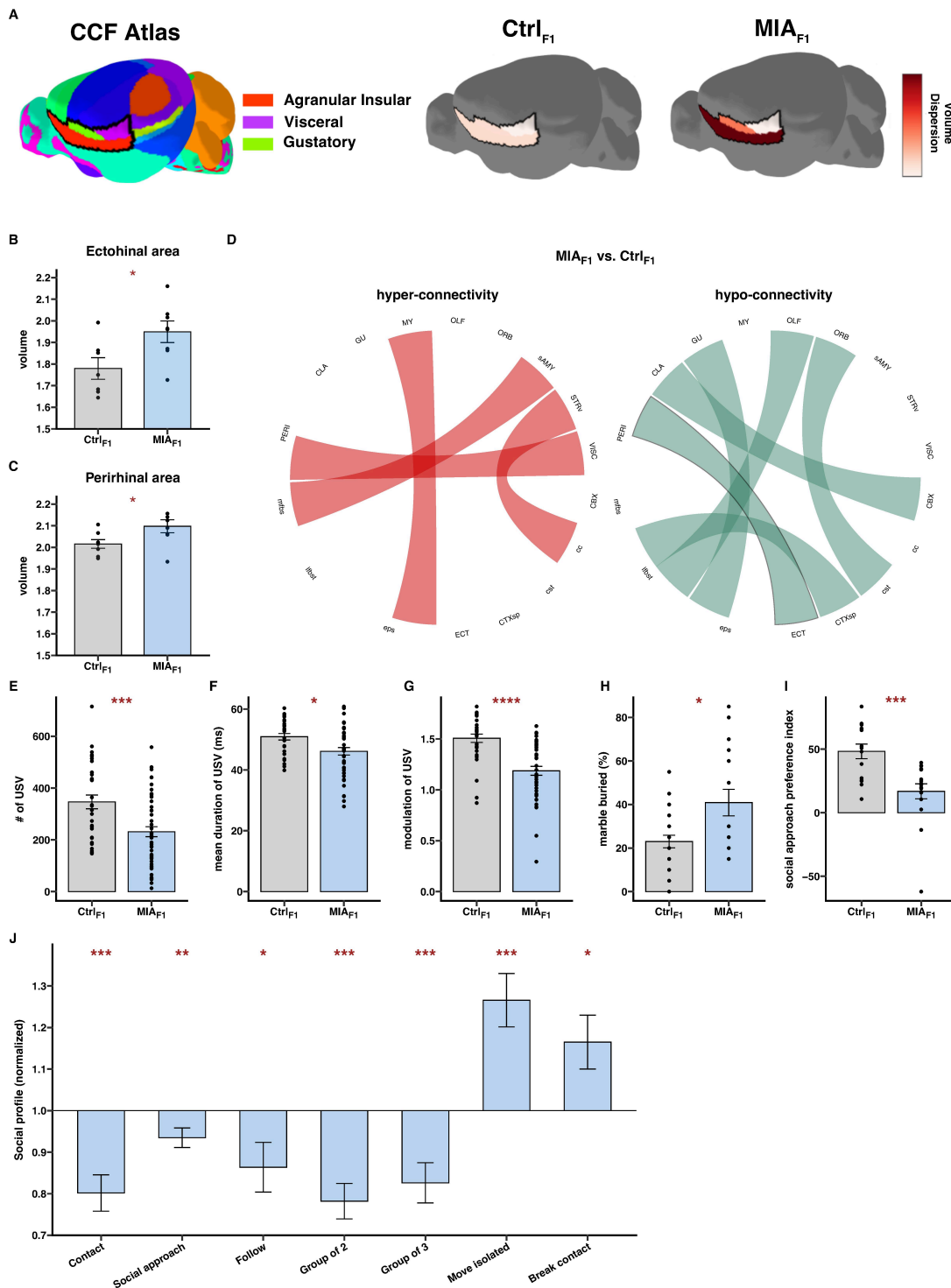


Figure 3

MIA disrupts brain morphology and connectivity and induces ASD-like phenotype in offspring.

(A) Cortical surface representation of the left hemisphere parcellated using the Allen Common Coordinate Framework (CCF) atlas. Regions implicated in autism-related circuitry - including the agranular insular, visceral and gustatory areas - are highlighted for reference. The surface map shows

the volume dispersion in these regions across Ctrl_{F1} **and** MIA_{F1} mice. Volume dispersion was calculated across mice within each group, revealing altered anatomical variability. **(B-C)** Brain volume of the (B) ectorhinal area and (C) perirhinal area in Ctrl_{F1} and MIA_{F1}. **(D)** Resting state functional connectivity showing hyperconnected regions (left) and hypoconnected regions (right) in MIA_{F1} compared to Ctrl_{F1}. **(E to G)** At day 7 (D7), pups were separated from their mothers to record USV. USV characteristics of Ctrl_{F1} (n = 32, 5 experiments), MIA_{F1} (n = 49, 7 experiments), showing the number of USV recorded within 5 min (E), mean USV duration (in ms) (F) and number of modulations per USV (G). **(H)** The marble buried test was assessed at week 6 (W6). Percentage of marbles buried in F1 mice, for Ctrl_{F1} (n = 25, 5 experiments), MIA_{F1} (n = 17, 4 experiments). **(I)** The F1 mice were then isolated for one week and the three-chamber test was performed at W7. Social preference index in F1 pups, for Ctrl_{F1} (n = 25, 5 experiments), MIA_{F1} (n = 17, 4 experiments) (H) Total exploration time during the three chambers. **(J)** The live mouse tracker (LMT) was recorded for 14 h at W8. Behavioral profiles of MIA_{F1} (n = 12) monitored over 14 hours of free interactions using a live mouse tracker (LMT) and normalised to Ctrl_{F1} mice (n = 12). LMT data were analysed using the Wilcoxon matched-pairs signed rank test. ACA, anterior cingulate area; AI, agranular insula area; CM, claustrum; ECT, ectorhinal area; GU, gustatory area; HY, hypothalamus; PERI, perirhinal area; sAMY, striatum-like amygdalar nuclei; STRd, striatum dorsal area; VIS, visceral area. Only males were used. Statistical analysis, t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Results are expressed as mean +/- SEM.

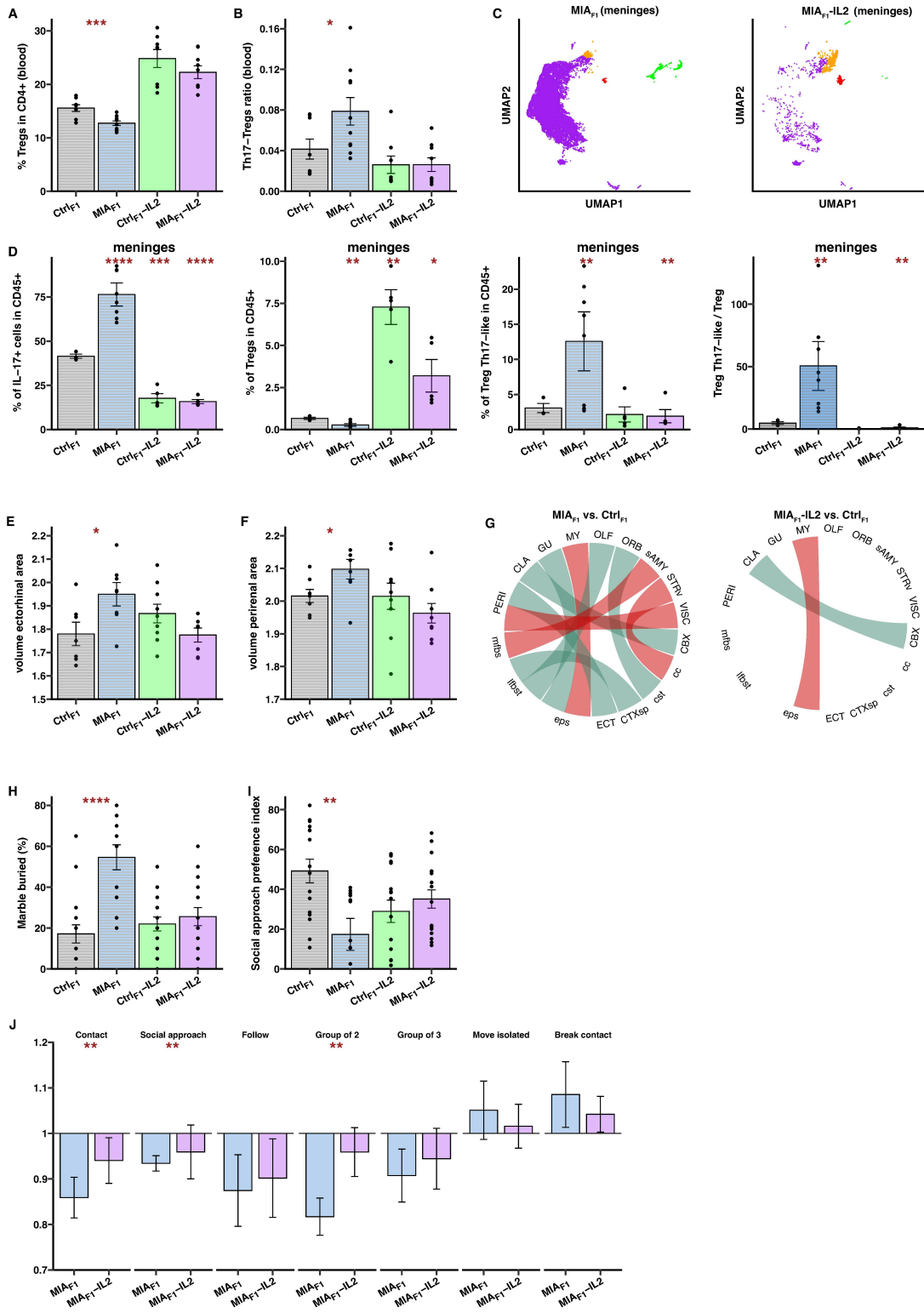


Figure 4

Postnatal treatment of MIA_{F1} mice with IL2_{LD} rescues ASD symptoms.

(A) Flow cytometry of Tregs at W5. **(B)** Th17/Tregs ratio at W5. **(C)** UMAPs of unsupervised flow cytometry from adult mice: MIA_{F1} (left) and MIA_{F1}-IL2 (right). **(D)** Cluster abundances based on UMAP analysis. **(E-F)** Brain volume of the (E) ectothal area and (F) perirhinal area in Ctrl_{F1}, MIA_{F1} and MIA_{F1}-IL2.

IL2 mice. **(G)** Resting state functional connectivity showing hyperconnected regions (red) and hypoconnected regions (green) in (left) MIA_{F1} compared to Ctrl_{F1} and (right) MIA_{F1}–**IL2** compared to Ctrl_{F1}. ACA, anterior cingulate area; AI, agranular insula area; CM, claustrum; ECT, ectorhinal area; GU, gustatory area; HY, hypothalamus; PERI, perirhinal area; sAMY, striatum-like amygdalar nuclei; STRd, striatum dorsal area; VIS, visceral area. **(H)** Percentage of buried marbles for Ctrl-**AAV_E** (n = 21, 4 experiments), MIA_{F1} (n = 13, 3 experiments) and MIA_{F1}–**IL2** (n = 17, 4 experiments) **(I)** Social preference index for Ctrl_{F1} (n = 21, 4 experiments), MIA_{F1} (n = 13, 3 experiments) and MIA_{F1}–**IL2** (n = 17, 4 experiments). **(J)** Live mouse tracker behavioural profiles for MIA_{F1} (n = 11) or MIA_{F1}–**IL2** (n = 10), normalised to the corresponding control mice (n = 12 per condition) over 14 h of free interactions. Only males were tested. Statistical analysis, t-test except for LMT: Wilcoxon matched-pairs signed rank test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Results are presented as mean +/- SEM. **Ctrl_{F1}: Ctrl_{F1} with empty control AAV injected at postnatal W3, MIA_{F1}: MIA_{F1} with empty control AAV; MIA_{F1}–IL2: MIA_{F1} with IL-2 producing AAV injected at postnatal W3.**

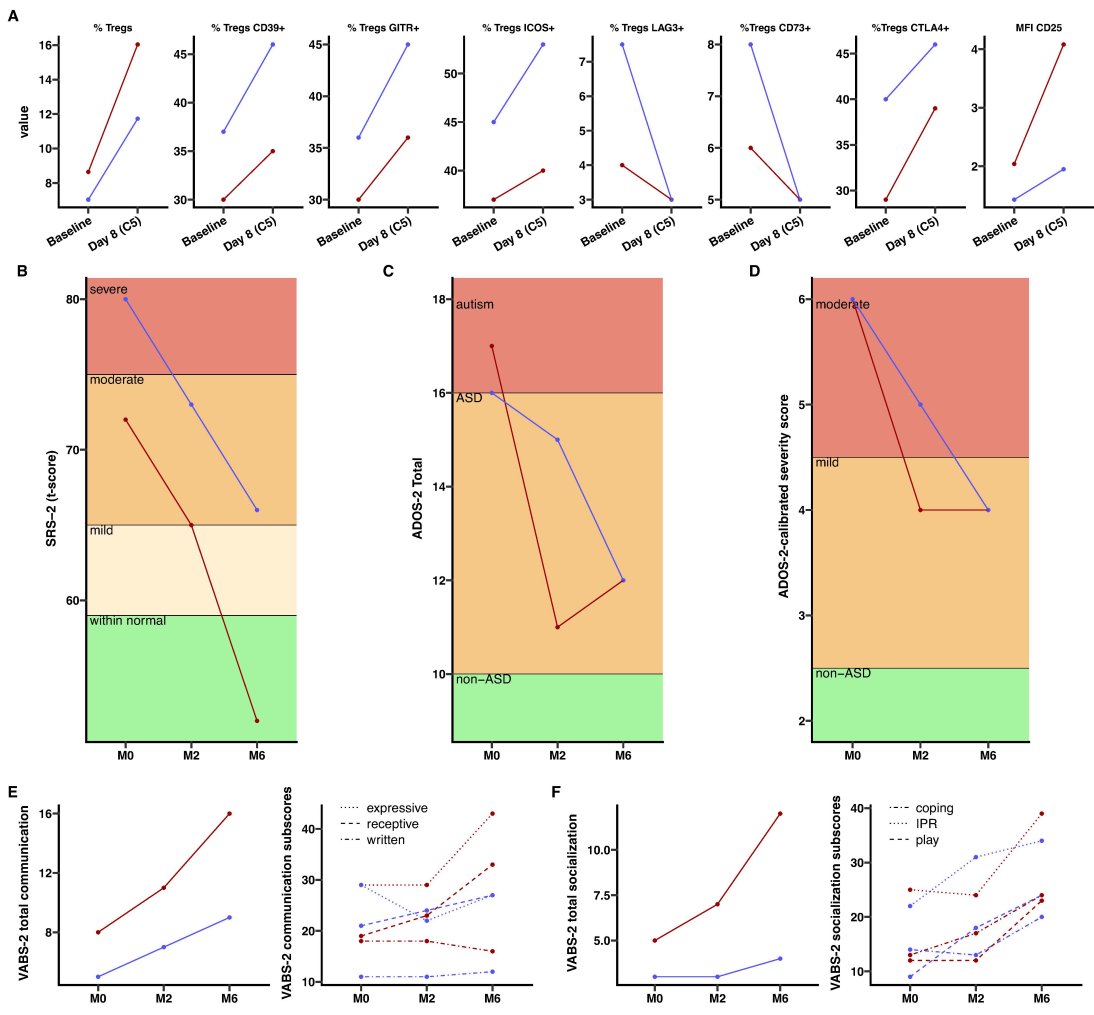


Figure 5

Longitudinal immunophenotypic and clinical changes in two children with ASD and a history of maternal immune activation after low-dose IL-2 treatment.

Patients received subcutaneous IL-2 at 0.8 MIU/m² for 5 consecutive days every 3 weeks for 6 months. **(A)** Changes in peripheral Treg phenotype between baseline and day 8 (during course-5), showing

frequencies of total Tregs, CD39+, GITR+, ICOS+, LAG3+, CD73+, and CTLA4+ Tregs, as well as CD25 mean fluorescence intensity (MFI). **(B–F)** Clinical evaluations were performed at baseline, 3 months, and 6 months. Behavioral and developmental assessments included the Social Responsiveness Scale, Second Edition (SRS-2), a caregiver-completed measure of social impairment; the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), a standardized semi-structured observational assessment of social communication and restricted and repetitive behaviors administered by trained clinicians specialized in autism spectrum disorder; and the Vineland Adaptive Behavior Scales, Second Edition (VABS-2), a caregiver interview assessing adaptive functioning in communication, socialization, and daily living skills. (B) SRS-2 T-score over time. (C) ADOS-2 total score over time. (D) ADOS-2 calibrated severity score over time. (E) VABS-2 communication total score over time (left), with subscores shown at right. (F) VABS-2 socialization total score over time (left), with subscores shown at right. Lines connect repeated measurements from the same participant across time points.

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