Embryo-restricted responses to maternal IL-17A promote neurodevelopmental disorders in mouse offspring

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Abstract

Prenatal imprinting to interleukin 17A (IL-17A) triggers behavioral disorders in offspring, but reported models lack specificity to elucidate the side of its action and the precise abnormalities it causes. By combining transgenic IL-17A overexpression with maternal deficiency in the receptor for IL-17A, we established a novel model to study embryo-restricted IL-17A responses. We demonstrated IL-17A transfer across the placental barrier and subsequent development of selected behavioral deficits in mouse offspring.

Introduction

Maternal immune activation (MIA) during pregnancy is a recognized risk factor for neurodevelopmental disorders (NDDs) in offspring[1,2,3]. NDDs require lifelong and resource-intensive specialized care with no cure up to date[4]. The prevalence of NDDs is increasing, underlining the importance of developing safe treatment strategies suitable for use during pregnancy to prevent NDDs in the first place[5]. Preclinical studies demonstrated that MIA, induced through treatment of the pregnant mouse female with the synthetic viral RNA analog Poly(I:C), results in imprinting of offspring to develop NDDs. In this model, interleukin-17A (IL-17A), a cytokine produced by a subset of maternal T helper 17 (TH17) cells, emerged as a central player in the impaired neurodevelopment[6,7]. Despite a prominent role of IL-17A in Poly(I:C)-MIA, this model does not allow to study the direct effects of IL-17A on embryonic development due to broad immune activation, induction of additional maternal-derived mediators beyond IL-17A (such as IL-6, IL-1β, among others), pregnancy complications, and batch-to-batch Poly(I:C) variability[7,8]. More importantly, discerning whether effects on the offspring stem from embryonic or maternal responses to Poly(I:C)-induced IL-17A is challenging. This clarification is pivotal for devising therapies, given the potential risk posed to maternal and fetal health by unintended drug effects.

Results and Discussion

The aim of our study was to establish a model of prenatal imprinting to maternal-derived IL-17A and to investigate embryo-restricted responses to maternal-derived IL-17A. To achieve this, we defined the following criteria for the model: 1) maternal blood should be enriched for IL-17A; 2) embryonic tissues should be responsive to IL-17A; 3) maternal tissues should be unresponsive to IL-17A; 4) pregnancy outcome and nursing should remain unaffected. To meet these criteria, we employed a genetic approach that minimized the need for mouse handling compared to pharmacological interventions, thereby reducing maternal stress during pregnancy. For that, we generated mice with constitutive IL-17A overexpression (OE) from a single copy transgene inserted into the ROSA26 locus while the second ROSA26 allele remained wildtype (WT). Additionally, we converted IL-17 receptor A (IL-17RA) in these mice to the complete knockout (KO) state (referred to as IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ mice). Deficiency in IL-17RA ensures unresponsiveness to IL-17A[9]. Subsequently, IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ females were paired with WT males (termed IL-17A^{OE} experimental mating) to produce offspring with two distinct genotypes: IL-17RA$^{WT/KO}$IL-17A$^{WT/WT}$ (short IL-17RA$^{KO/WT}$) and IL-17RA$^{WT/KO}$IL-17A$^{OE/WT}$, both responsive to IL-17A due to one functional IL-17RA allele inherited from the father (Fig.1a). Intrinsic IL-17A overexpression on a IL-17RA sufficient background leads to moderate-to-lethal inflammation[10,11] and therefore IL-17A transgenic IL-17RA$^{WT/KO}$IL-17A$^{OE/WT}$ offspring were not considered for the purpose of the present study. Instead, we evaluated IL-17RA$^{KO/WT}$ offspring without IL-17A

transgene which were exposed to maternal-derived IL-17A *in utero*, allowing us to unequivocally study the direct role of prenatal imprinting to IL-17A. To generate appropriate controls for IL-17A-imprinted offspring, IL-17RA$^{KO/KO}$ females without IL-17A transgene were paired with WT males (termed control mating). This mating yielded genotype-matched IL-17RA$^{WT/KO}$ offspring that developed in the absence of transgenic IL-17A in dams (Fig.1a). Consequently, we performed a set of pre- and postnatal analyses at different time points to determine the effect of embryonic IL-17A imprinting on pregnancy outcome, immune system homeostasis, and behavior (Fig.1b). As designed, sera of dams in experimental IL-17A^{OE} matings displayed high levels of IL-17A (Fig.1c). Importantly, no such elevation was observed in the levels of IL-6 (Fig.1d), which is also a critical cytokine for NDD development[7].

Since complications during pregnancy can lead to behavioral disorders in offspring[12,13], we evaluated pregnancy progression in our model. Our analysis revealed that high levels of IL-17A in dams devoid of IL-17A signaling does not affect the number of implanted and resorbed embryos (Fig.1e-f). Litter sizes remained unaffected and the body weight of IL-17A-imprinted pups at wean did not differ from controls (Fig.1g-h). Furthermore, IL-17RA$^{KO/WT}$ embryos born to IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ females exhibited unaltered morphology, body- and placental weight at E13.5 (Fig.1i-j). Taken together, embryonic IL-17A responses in IL-17RA deficient dams did not result in adverse pregnancy outcomes.

In humans and mice, the placenta strictly separates maternal and embryonic blood[14,15]. To test whether maternal-derived IL-17A crosses the placenta, we evaluated IL-17A levels within embryonic tissues and fluids. We revealed elevated IL-17A levels in placentas, embryonic membranes, and amniotic fluid of IL-17RA$^{KO/WT}$ embryos in IL-17A^{OE} matings, as compared to controls (Fig.1k-m). Thus, as designed, embryos in our experimental IL-17A^{OE} matings are exposed to maternal-derived IL-17A and represent a suitable model to study embryo-restricted IL-17A responses.

A disrupted gut microbiota homeostasis in dams promotes behavioral abnormalities in offspring exposed to MIA[6,16,17]. To consider the potential impact of the maternal microbiota on prenatal imprinting in our model, we used IL-17RA$^{KO/KO}$ and IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ dams which were littermates and were continuously co-housed prior to mating set up. Co-housing is a useful strategy to ensure aligned microbiota composition in mice[18]. This approach allowed us to rule out potential effects related to differences in maternal gut microbiota and attribute findings solely to embryonic IL-17A responses. Imprinting resulting from maternal infection can alter immune responses in the gut of offspring[19,20]. This way, maternal-derived IL-6 can act on embryonic intestinal stem cells, thereby leading to persistent changes in the TH17 cell population in adulthood[20]. To explore a potential prenatal imprinting effect of IL-17A on T cell homeostasis, we assessed RORγt^{+} TH17 cells, Foxp3^{+} regulatory T (Treg) cells as well as gut-associated RORγt^{+}Foxp3^{+} Treg cells in IL-17RA$^{KO/WT}$ offspring born from control and IL-17A^{OE} matings. We observed no variations in the distribution or quantity of the aforementioned T cell subsets, neither in the small intestine, nor in the colon or spleen (Fig.2 and Supplementary Fig.1). Consequently, we found no indication for imprinting effects of maternal IL-17A on the intestinal and systemic TH17/Treg cell homeostasis.

Next, we assessed whether embryo-restricted IL-17A signaling impacts behavior of offspring. Therefore, we first evaluated the ultrasonic vocalization profile of pups at postnatal day 9. IL-17A-imprinted pups showed reduced number of vocalizations compared to control offspring (Fig.3a). The communicative irregularities in early life led us to conduct behavioral tests with adult mice. To assess anxiety-like behavior, we performed an elevated plus maze test and found no differences

in the time spent in open or closed arms in male offspring born from IL-17A^{OE} matings (Fig.3b). However, the open-field test revealed a reduction in the time spent in the center area in IL-17A-imprinted mice (Fig.3c). This effect did not result from changes in activity as the distance mice traveled during the test remained similar to controls (Fig.3c). We concluded that prenatal exposure to IL-17A may lead to anxiety-like behavior in the experimental settings. No differences were noted in the time spent with inanimate objects or unfamiliar mice during a social approach test. Similarly, a social novelty test did not reveal differences in the interaction time with familiar or novel mice (Fig.3d-e). These results pointed that social behavior was not affected in IL-17A-imprinted offspring. Finally, a reduction in repetitive behavior was observed in the marble burying test, indicating a reduction in repetitive behavior (Fig.3f). Collectively, these data show that embryonic IL-17A signaling alone is sufficient to cause a selective set of behavioral disturbances. The absence of immune system alterations in IL-17A imprinted offspring suggests that the embryonic brain is the primary target for maternal-derived IL-17A. Previous reports showed that IL-17RA deletion in neurons of adult mice, driven by Syn1-Cre, impacts behavior[21,22]. Another study suggested that IL-17A acts on the developing brain[7], but mechanistic evidence for IL-17A signaling in embryonic brain neurons is limited. Follow up studies should reveal IL-17A responsive cell types in the embryonic brain critical for the development of behavioral abnormalities.

Here, we found no evidence that embryonic IL-17A responses cause pregnancy complications, which contrasts to growth retardation observed in offspring born to WT dams treated with IL-17A[23]. However, unlike in our study, WT dams were IL-17A responsive and experienced handling stress which may explain differences in pregnancy outcomes. Previous studies reported social behavior impairments following maternal Poly(I:C) treatment[6,7]. Intriguingly, maternal IL-17RA deficiency prevented Poly(I:C)-induced disturbances in social behavior[7]. Likewise, we found no alterations in social behavior in IL-17A imprinted offspring born to IL-17A^{OE} dams with IL-17RA deficiency. These reported findings, together with ours, suggest that sociability in offspring is shaped by maternal rather than embryonic responses to IL-17A. In contrast, embryonic responses alone are sufficient to drive anxious behavior during the open field test, which overlap between Poly(I:C)-MIA and our genetic model. Opposing effects were observed in other behavioral tests. Poly(I:C)-MIA offspring emitted more ultrasonic vocalizations than controls, while IL-17A imprinted offspring from our model emitted fewer. Similarly, Poly(I:C)-MIA offspring bury more marbles, while IL-17A imprinted mice bury fewer[6,7]. These contradictory results might stem from differences in IL-17A dosage and exposure timing. Poly(I:C) is typically injected at midgestation, while our model is characterized by a chronic and rather high IL-17A levels which supposedly may act on embryos starting from a very early developmental stage. Further, additional factors induced by Poly(I:C), like IL-6, might have synergistic effects with IL-17A, which would result in distinct behavioral outcomes.

Taken together, our innovative mouse model of prenatal imprinting to maternal IL-17A, allows to study embryonic responses to a single stress factor, IL-17A. To our knowledge, this is the first study that detected maternal-derived IL-17A within the amniotic fluid and extra-embryonic tissues of the developing fetus. This implies that maternal IL-17A passes through the placental barrier and this passage is likely a critical mechanism through which IL-17A imprints a selected set of behavioral deficits (Supplementary Fig.2). Our findings suggest that targeting the embryonic IL-17A pathway during pregnancy may prevent some forms of neurodevelopmental disorders, such as abnormalities in vocalization, anxiety-like and repetitive behavior.

Methods

Mice and colony maintenance

All mice were on C57BL/6 background. Wild type (WT) mice were bred in-house. IL-17RA knockout (IL-17RA$^{KO/KO}$) mice were generated by crossing IL-17RA floxed mice[24] with CMV-Cre mice[25]. Next, mice were intercrossed with IL-17A inducible (IL-17A^{ind}) mice[11] to generate IL-17RA$^{KO/KO}$IL-17A$^{ind/WT}$CMV-Cre$^{+/WT}$ mice. These mice achieved constitutive germline IL-17A overexpression (IL-17A^{OE}) on IL-17RA$^{KO/KO}$ background. In the following intercrosses CMV-Cre was crossed out resulting in IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ mice. To generate mice for control and experimental mating (Fig.1a), as well as for colony maintenance, IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ mice were bred with IL-17RA$^{KO/KO}$ mice.

All mice were bred and maintained under specific-pathogen-free (SPF) conditions with food and water ad libitum in a 12h light/12h dark cycle with controlled room temperature and humidity at the Translational Animal Research Center (TARC) of the University Medical Center Mainz. After weaning, sex-matched littermates were co-housed until further use. To control for environmental factors, all breedings were conducted in the same area of the animal facility. Ethical approval was obtained from the Landesuntersuchungsamt Koblenz (G22-1-003), and all animal experiments followed the guidelines of the German Animal Welfare Act.

Genotyping

Tissue biopsies were incubated in lysis buffer (Tris-HCl pH=8, KCl, EDTA, Igepal, Tween20 in ddH_2O) supplemented with Proteinase K (7BioScience) at 56°C on a shaker overnight. The digested tissue was incubated for 5min at 95°C, followed by centrifugation (12000rcf, 10min, RT). The resulting supernatant was used for genotyping through REDTaq PCR Reaction Mix (Sigma Aldrich). Specific primer sets were applied to detect the CMV-Cre and IL-17A transgenes, IL-17RA^{WT} and IL-17RA^{KO} alleles, and to determine the configuration of the Gt(ROSA)26Sor locus targeted with the IL-17A transgene.

Timed pregnancies and litter size evaluation

One or two virgin females (8-10wks) were paired with one wildtype male in the evening (6-9pm). The presence of a vaginal plug in the morning (7-10am) indicated copulation and marked gestational day (E) 0.5. Females with mating plugs were separated from males and were housed alone or with a companion female. Pregnant females at term were inspected in the mornings to evaluate litter sizes.

Maternal serum collection and prenatal analysis

Pregnant mice were sacrificed at E13.5 or E16.5 through deep anesthesia followed by cardiac blood withdrawal. Blood was incubated at room temperature for 30 min and centrifuged (12000rcf, 20min, RT) for serum collection. Sera were stored at -80°C until used for IL-17A measurements. Uteri were extracted and washed with DPBS$^{-/-}$ (Gibco) for assessment of embryo implantations and resorptions.

Embryonic tissue collection

Individual embryos with their amniotic sac intact were dissected, washed with DPBS$^{-/-}$, and positioned on clean petri dishes. Amniotic fluid was collected by puncturing the amniotic sac with single-use 30G insulin syringes (BD Biosciences), followed by subsequent centrifugation (300rcf, 7min, 4°C) and collection of the cell-free supernatant. Placentas and embryonic membranes (pooled umbilical cord and amniotic sac) were separated from the embryo. Embryos, placentas, and membranes were dried on paper towels before being weighted. Deciduas were mechanically removed from placentas. The collected amniotic fluid and extra-embryonic tissues were stored at -80°C until used for IL-17A measurements.

Tissue processing and ELISA

Extra-embryonic tissues were lysed in RIPA buffer (Tris-HCl pH=7.5, NaCl, EDTA, Igepal, Sodium Deoxycholate in ddH_2O) supplemented with protease- (cOmplete™, Sigma-Aldritch) and phosphatase-(PhosSTOP™, Roche) inhibitors using bead tubes (MP Biomedicals). After homogenization and centrifugation (12000rcf, 10min, 4°C), the debris-free supernatant was collected. Cytokine concentrations in sera, amniotic fluid, and tissue homogenates were measured using an IL-6 (BD #555240) or IL-17A ELISA kit (Invitrogen #88-7371) according to the manufacturers recommendations. Concentrations were calculated with standard curves and final concentrations were normalized to tissue weight. Concentrations for background subtracted OD values below 0 were defined as 0pg/ml.

Ultrasonic vocalization test with pups

The day of birth was considered as postnatal day 0 (P0). On P9, breeding cages containing dam with litter were transferred to the testing room and habituated for 30min. One pup at a time was individually separated from the nest and placed in a clean beaker within a light-proof Styrofoam box and vocalization profiles were recorded for 3min using a Avisoft Bioacoustic UltraSoundGate 116Hb recorder with condenser microphone. After measurement, the pup was returned to its nest. The beaker was cleaned between each pup.

Behavioral tests with adult mice

Tests with adult mice were conducted and analyzed at the Mouse Behavior Unit (MBU) of the Leibniz Institute for Resilience Research (LIR) gGmbH, Mainz, Germany. Male mice at 8-10wks were transferred in separated cages for single-housing in the testing facility. There, mice acclimated for at least one week to the new environment. During this period, mice were accustomed to the experimenter through repeated handling. For each test, mice were introduced to the testing room and acclimated for 30min before being gently transferred to the test area (RT, 70 Lux, 9am-6pm) via tunnel or cup handling. Except for the marble burying test, all tests were video-recorded and subjected to automated analysis. The testing areas were cleaned between individual mice. At least one day of rest separated consecutive tests. The sequence of tests followed the description below.

Elevated plus maze test

A mouse was placed at the intersection point of an elevated X-shaped maze (75cm above the ground). It consisted of two arms (77cm x 7cm), one with walls (20cm high, closed arm), and the other without (open arm). The mouse explored both arms for 10min, and time spent and number of entries into each arm were quantified.

Three-chamber sociability and social novelty test

After a 10-minute habituation in an empty test arena (63cm x 41cm x 22cm) with three chambers (21cm x 41cm x 22cm each), the test mouse was placed in the central chamber. Additionally, an unfamiliar adult wildtype male mouse was introduced in one of the side chambers, while an inanimate object was placed in the other. During a 10-minute period, the test mouse interacted with both the mouse and object. Interaction time with the mouse and object was quantified. This process was repeated with a novel (unfamiliar) male mouse replacing the object. Interaction time with familiar and novel mice was quantified. Unfamiliar mice were used for up to three consecutive encounter with experimental mice.

Open field test

A mouse was introduced into an empty test arena (42cm x 42cm x 41cm) and allowed to explore for 10min. The time spent in a defined center area (10cm x 10cm) was quantified.

Marble burying test

A mouse was placed in a test area (22cm x 38cm x 20cm) filled to a depth of 5cm with bedding. On top of the bedding, 16 marbles were placed in a specific pattern (4 marbles per row, 4 rows). The test mouse was free to explore the cage and dig in the bedding. After 30 minutes, the mouse was removed, and the number of fully buried marbles was counted.

Leukocyte isolation from spleen and intestinal lamina propria

Colons and small intestines (without caecum) from female mice (8-20wks) were isolated and transferred into RPMI-1640 (Gibco) medium supplemented with L-glutamine, Penicillin/Streptomycin (Pen/Strep), FCS and HEPES (hereafter referred to as 3% medium). Peyer's patches were removed, and intestines were cleaned with 3% medium before incubation (37°C, 20min, horizontal shaker) with strip medium (3% medium supplemented with EDTA and DTT). Samples were vortexed rigorously and strained. The tissue was transferred into RPMI-1640 medium supplemented with L-glutamine, Pen/Strep, EDTA, and HEPES, vortexed rigorously, strained and washed with 3% medium. Then intestines were mechanically dissociated and incubated (37°C, 30min, horizontal shaker) in digestion medium (RPMI-1640 supplemented with L-glutamine, Pen/Strep, β-ME, HEPES, Sodium Pyruvate, Liberase TL™ (Sigma Aldritch) and DNase I). Then, the cell suspension was vortexed and passed through a 100µm filter. Finally, cells were centrifuged (450g, 10min, 4°C), resuspended in 3% media and kept on ice until further use. Spleens were mechanically dissociated through a 40µm filter and resuspended in $DPBS^{-/-}$ with 2%v/v FCS (hereafter referred to as PBS/FCS). Cells were centrifuged (300rcf, 7min, 4°C) and resuspended in 1ml Ammonium-chloride-potassium lysis buffer to remove erythrocytes. After 1min incubation at RT, cells were washed, centrifuged (300rcf, 7min, 4°C) and resuspended in PBS/FCS.

Flow cytometry

Cells were centrifuged (300rcf, 7min, 4°C), resuspended in PBS/FCS supplemented with Fc-block (BioXCell) and incubated for 15min on ice. After, cells were centrifuged (300rcf, 7min, 4°C), resuspended in PBS/FCS containing fixable viability dye (APC-eFl780, eBioscience #65-0865, 1:1000) and antibodies directed against surface molecules CD11b (biotinylated, eBioscience #13-0112, 1:400), F4/80 (PE, eBioscience #12-4801, 1:50), CD4 (BV510, Biolegend #100559, 1:200), TCRβ (FITC, Biolegend #109205, 1:200). After 15min incubation on ice, cells were centrifuged (300rcf, 7min, 4°C) and fixed (1h, 4°C) using the eBioscience Foxp3/Transcription factor staining buffer set (ThermoFisher #00-5523-00) fixation/permeabilization reagent. After incubation, cells were centrifuged (350rcf, 7min, 4°C) and resuspended in permeabilization buffer containing Streptavidin (PerCP, Biolegend #405213, 1:600) and antibodies directed against intracellular targets Foxp3 (APC, eBioscience #17-5773, 1:200) and RORγt (BV421, BD Biosciences #562894, 1:200). After 1h incubation at 4°C, cells were centrifuged (350rcf, 7min, 4°C), resuspended in PBS/FCS and acquired on a BD FACSCanto II flow cytometer.

Statistical analysis and data presentation

Discrete datasets were analyzed using the two-tailed Mann-Whitney test to determine statistical significance, while continuous datasets with a presumed normal distribution underwent analysis via the two-tailed unpaired t-test. Welch's correction was applied when significant differences in variance were present between groups. Data were presented either as median±95% confidence interval (CI) or mean±standard deviation (SD), depending on whether the data were discrete or continuous. Statistical significance levels were indicated as follows: ns=$p>0.05$, *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$ and ****=$p<0.0001$. Behavioral test results involving adult mice were presented using box and whisker plots, encompassing 2.5-97.5 percentiles and min/max values, with a "+" indicating the mean. Outlier were not excluded from the analysis.

Software

Avisoft SAS Lab Pro software 5.1 was used for ultrasonic vocalization profile recording, and Sonic Visualizer v4.3 was employed for their subsequent analysis. EthoVision XT by Noldus was used for automated analysis of behavioral test video recordings. Graphs were generated, and statistical analyses were performed using GraphPad Prism 9. Illustrations were created using CorelDRAW Graphics Suite 2022. Microsoft 365 (Office) tools, specifically Word for manuscript writing, Excel for data analysis, and Publisher for figure design, were used.

Data availability

Data used for graphs, raw data and statistical analysis can be provided upon reasonable request.

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Contributions

D.A. and I.A.M. designed experiments, performed timed pregnancies, tissue collections, and analyzed data. D.A. and M.B. performed ELISA and flow cytometry experiments. D.A. performed and analyzed behavioral tests. M.B., C.S., D.C.U. and L.S. assisted with animal husbandry, timed pregnancies, and mice genotyping. K.R. advised on behavioral test design and provided equipment for behavioral tests. A.W. provided funding and mice. I.A.M. conceptualized and supervised the study. D.A. wrote the manuscript (original draft, review, and editing). A.W. and I.A.M. wrote the manuscript (review and editing). All authors contributed to editing the manuscript.

Conflict of Interest

The authors declare no competing interests

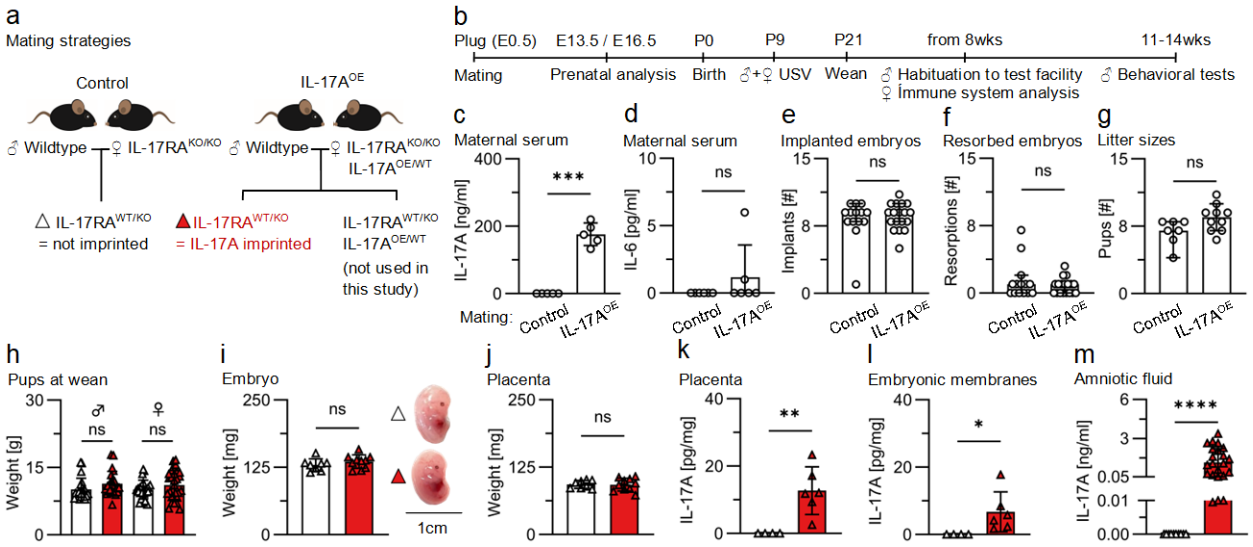


Fig.1 Analysis of a genetic model of maternal IL-17A overexpression combined with IL-17RA deficiency for pregnancy progression and IL-17A passage through placenta a) Mating scheme to achieve prenatal imprinting to maternal-derived IL-17A. **b)** Experimental timeline of the study. Dams were sacrificed for prenatal analysis or allowed for natural delivery. At postnatal day 9 (P9), pups were subjected to an ultrasonic vocalization test (USV). At P21, pups were weaned. Female offspring were sacrificed for immune system analysis, starting from 8wks of age. Male offspring, from 8wks of age, were single-housed, transferred to the behavioral test facility for habituation and further subjected to behavioral tests. **c)** Maternal IL-17A serum concentrations at E13.5. n=5 dams per group. **d)** Maternal IL-6 serum concentrations at E13.5. n=6 dams per group. **e)+f)** Number of implanted and resorbed embryos in indicated mating types. Data pooled from timepoints E13.5 and E16.5. n=14 litters in control mating and 18 litter in IL-17A^{OE} mating. **g)** Number of offspring born in indicated mating types. n=7 litters in Control and 10 litters in IL-17A^{OE} mating. **h)** Body weight of pups at wean. n=21 (males) and 23 (females) from 7 litters in control mating (white), and 22 (males) and 29 (females) from 12 litters in IL-17A^{OE} mating (red). **i)+j)** Body and placenta weight of embryos at E13.5. n=8 from 1 litter in control mating (white) and 10 from 2 litters in IL-17A^{OE} mating (red). **k)+l)** IL-17A levels in placental and embryonic membrane (pooled umbilical cord and amniotic sac) tissue lysate at E13.5. n=4 from 1 litter in control mating (white) and 6 from 2 litters in IL-17A^{OE} mating (red). **m)** IL-17A levels in the amniotic fluid at E13.5. n=10 from 5 litters in control mating (white) and 24 from 13 litters in IL-17A^{OE} mating (red).

c)-g) Data points represent individual dams. Control=IL-17RA$^{KO/KO}$ dams and IL-17A^{OE}=IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ dams mated with Wildtype males. h)-m) Data points represent individual pups or embryos. e)-g) Bar graphs indicate median±95% CI. c)+d),h)-m) Bar graphs indicate mean±SD.

c)+d),k)-m) ****=p<0.0001, ***=p<0.001, **=p<0.01, *=p<0.05, ns=p>0.05 two-tailed unpaired t-test with Welch's correction. e)-g) ns=p>0.05 two-tailed unpaired Mann-Whitney test. h)-j) ns=p>0.05 two-tailed unpaired t-test.

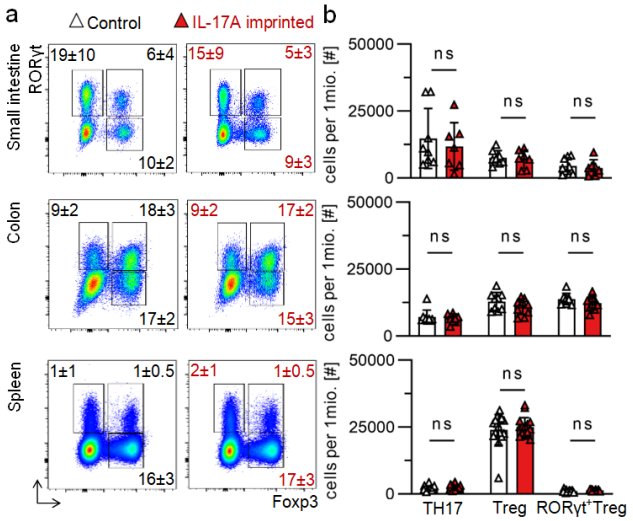
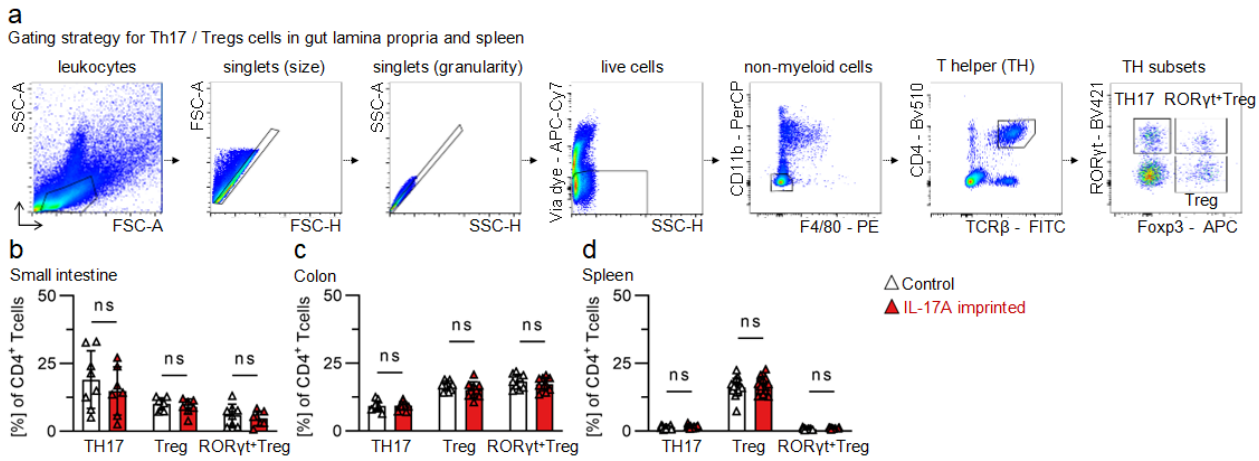


Fig.2 TH17 and Treg cells are not impacted in adult offspring prenatally exposed to IL-17A. a) Representative FACS plots depict RORγt and Foxp3 expression (gated on: CD11b$^-$F4/80$^-$CD4$^+$TCRβ$^+$ cells) and **b)** numbers of TH17, Treg and RORγt$^+$ Treg cells in the small intestine (top), colon (middle) and spleen (bottom). Quantifications show pooled data from two (intestines) and four (spleen) independent experiments performed with female mice. To normalize data from independent experiments, the cell number of each T cell subset was divided by the number of million cells in the sample. Small intestine: n=8 from 2 litters (white) and 7 from 3 litters (red); Colon: n=9 from 4 litters (white) and 10 from 5 litters (red); Spleen: n=17 per group from 6 litters (white) and 8 litters (red).
Datapoints represent individual mice. Bar graphs indicate mean±SD. ns=p>0.05 two-tailed unpaired t-test. Control=IL-17RA$^{KO/WT}$ offspring born to IL-17RA$^{KO/KO}$ dams (white). IL-17A imprinted=IL-17RA$^{KO/WT}$ offspring born to IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ dams (red).



Supplementary Fig.1 Gating strategy and TH17/Treg cell frequencies. a) Gating strategy for identification of intestinal lamina propria and splenic TH17/Treg/RORγt+Treg cells by flow cytometry. **b)-d)** Frequencies of indicated cells types among CD4$^+$TCRβ$^+$ cells in the small intestine (b), colon (c) and spleen (d). Quantifications show pooled data from two (intestines) and four (spleen) independent experiments performed with female mice. Small intestine: n=8 from 2 litters (white) and 7 from 3 litters (red); Colon: n=9 from 4 litters (white) and 10 from 5 litters (red); Spleen: n=17 per group from 6 litters (white) and 8 litters (red).
Datapoints represent individual mice. Bar graphs indicate mean±SD. ns=p>0.05 two-tailed unpaired t-test. Control=IL-17RA$^{KO/WT}$ offspring born to IL-17RA$^{KO/KO}$ dams (white). IL-17A imprinted=IL-17RA$^{KO/WT}$ offspring born to IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ dams (red).

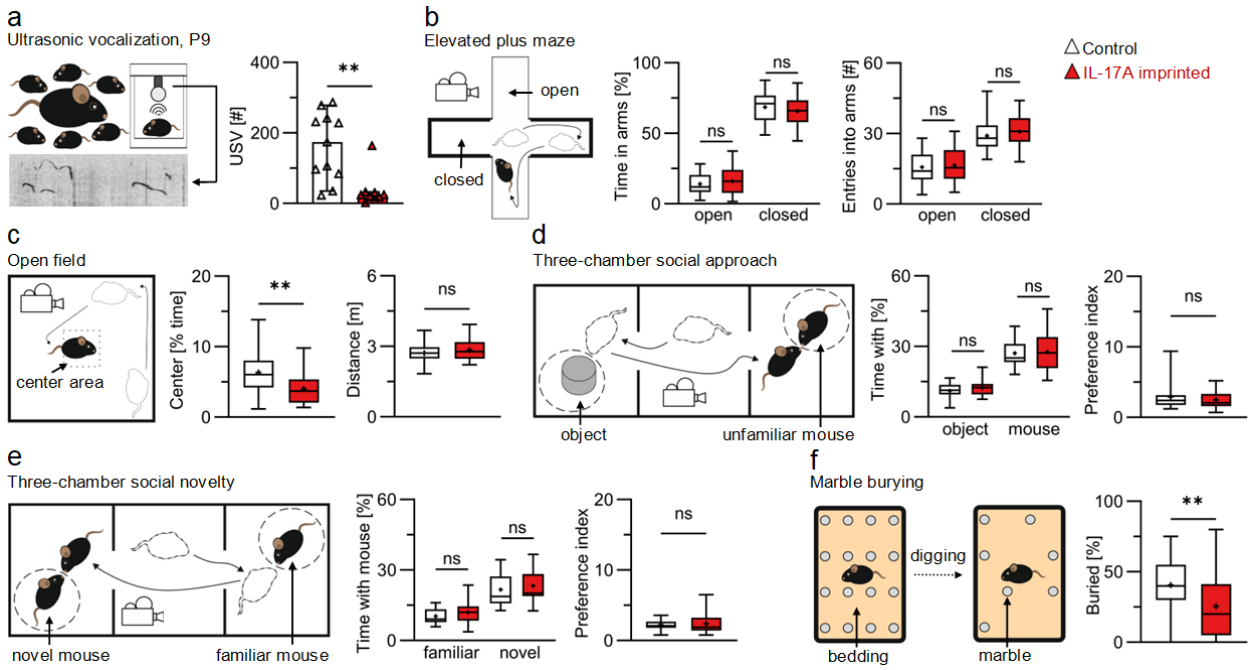
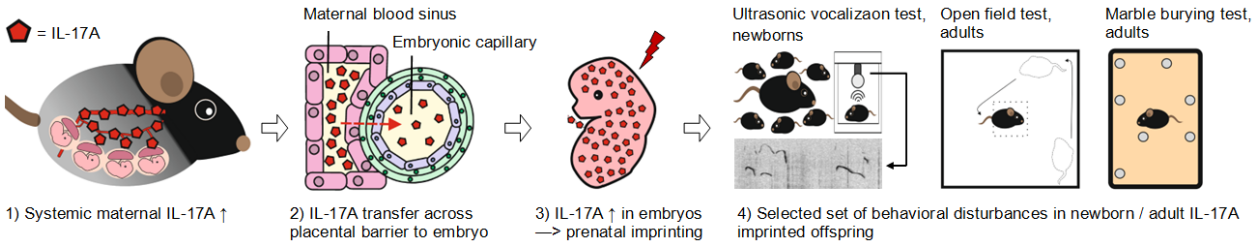


Fig.3 Embryo-restricted IL-17A responses lead to behavioral disturbances a) Ultrasonic vocalization test. Number of ultrasonic vocalizations (USV) emitted in the test period. Datapoints represent individual pups of mixed sex. Bar graphs represent median±95%CI. n=11 from 2 litters (white) and 9 from 3 litters (red). **b)** Elevated plus maze test. Left: Time spent in open and closed arms. Right: Number of entries into open and closed arms. **c)** Open field test. Left: Time spent in the center area. Right: Total distance moved within the test period. **d)** Three-chamber social approach test. Left: Time spent with an inanimate object and an unfamiliar mouse. Right: Preference index as ratios of time spent with an unfamiliar mouse to time spent with an inanimate object. **e)** Three-chamber social novelty test. Left: Time spent with novel and familiar mice. Right: Preference index as ratios of time spent with an unfamiliar mouse to time spent with a familiar mouse. **f)** Marble burring test. Frequency of buried marbles within the test period.
a)+f) **=p<0.01 two-tailed unpaired Mann-Whitney test. b)-e) **=p<0.01, ns=p>0.05 two-tailed unpaired t-test. b)-f) Boxes indicate 2.5-97.5 percentile. Whiskers indicate Min./Max. values. Horizontal lines indicate median. "+" indicates mean. n=21 from 7 litters (white) and 22 from 12 litters (red). Pooled data from 4 independent test cohorts. Males only, 11-14wks. Graphics on the left show the experimental setup of an individual test. Control=IL-17RA$^{KO/WT}$ offspring born to IL-17RA$^{KO/KO}$ dams (white). IL-17A imprinted=IL-17RA$^{KO/WT}$ offspring born to IL-17RA$^{KO/KO}$IL-17A$^{OE/WT}$ dams (red).



Supplementary Fig.2. Proposed mechanism by which the excess of maternal IL-17A mediates behavioral disturbances in offspring. IL-17A present in the maternal blood (1) is translocated across the placental barrier (2) which is formed by four embryonic cell layers (Pink=Trophoblast giant cell layer; Green=Syncytiotrophoblast bilayer; Blue=Endothelial cell layer). Maternal-derived IL-17A is distributed within the embryo, leading to prenatal imprinting to IL-17A (3). Exposure to IL-17A in the prenatal period leads to long-lasting behavioral disturbances (4).