HHS Public Access

Author manuscript

J Neuroimmunol. Author manuscript; available in PMC 2021 February 15.

Published in final edited form as:

J Neuroimmunol. 2020 February 15; 339: 577118. doi:10.1016/j.jneuroim.2019.577118.

The contribution of cyclophilin A to immune-mediated central nervous system inflammation

Vahnee Garimella, **Julie Secor McVoy**, **Unsong Oh**

Department of Neurology, Virginia Commonwealth University School of Medicine, P.O. Box 980599, Richmond, VA 23298, USA

Abstract

Cyclophilin A has multiple known functions in inflammation. Intracellular cyclophilin A modulates T helper 2 response (Th2) and extracellular cyclophilin A functions as a leukocyte chemotactic factor. The contribution of cyclophilin A to central nervous system (CNS) inflammation has not been reported. To test the hypothesis that inhibition of cyclophilin A would ameliorate immune-mediated CNS inflammation, we compared the course and neuroimmunology of experimental allergic encephalomyelitis (EAE) in cyclophilin A knockout mice and wild type littermates. There was a trend towards lower incidence of EAE in cyclophilin A knockout mice, but the clinical course of EAE among animals that manifested clinical signs of EAE was similar in cyclophilin A knockout and wild type littermates. Antigen recall response to myelin oligodendrocyte glycoprotein (MOG) peptide showed that interferon-γ release was lower in cyclophilin A knockout mice. Analysis of CNS inflammatory cells showed that CD3+ T cell infiltration into the CNS was lower in cyclophilin A knockout mice. These results showed that the loss of cyclophilin A results in altered peripheral immune activation and CNS leukocyte infiltration, but these changes did not result in a substantial change in the clinical course of EAE.

Keywords

cyclophilin A; experimental allergic encephalomyelitis; central nervous system inflammation

1. Introduction

Cyclophilin A, also known as peptidylprolyl isomerase A (PPIA), is an 18 kilodalton protein that catalyzes cis-trans isomerization at proline imidic peptide bonds, thereby promoting protein folding/trafficking and regulating protein activity (Takahashi et al., 1989). Cyclophilin A has multiple known functions in inflammation. Intracellularly, cyclophilin A interacts with interleukin (IL)-2 inducible T cell kinase (ITK) to tune T cell receptor signaling (Brazin et al., 2002). Cyclophilin A modulates ITK function, and the loss of

Corresponding author: Unsong Oh, MD. uoh@vcu.edu.

Conflict of interest: none

cyclophilin A results in derepression, in particular, of Th2 response (Colgan et al., 2004). Extracellularly, cyclophilin A is known to function as a leukocyte chemotactic factor. Cells secrete cyclophilin A by a vesicular secretory pathway in response to lipopolysaccharide and oxidative stress (Sherry et al., 1992; Suzuki et al., 2006), or cyclophilin A may be released during cell death (Christofferson and Yuan, 2010; Dear et al., 2011). Chemotactic function of cyclophilin A is known to involve interaction with extracellular matrix metalloproteinase inducer (EMMPRIN or CD147), activating matrix metalloproteinases (Yurchenko et al., 2002). The chemotactic function of extracellular cyclophilin A is a reliable finding across species, acting on monocytes/macrophages, human neutrophils, human T cells and eosinophils (Damsker et al., 2007; Heinzmann et al., 2015; Xu et al., 1992). By inhibiting cyclophilin A, leukocyte infiltration and disease activity were reduced in a number of animal models of inflammation including models of allergic lung injury, biliary atresia and allergic myocarditis (Arora et al., 2005; Balsley et al., 2010; Heinzmann et al., 2015; Iordanskaia et al., 2015). Thus prior knowledge indicates that cyclophilin A influences inflammatory responses through its actions on immune activation and/or leukocyte trafficking.

Mechanisms of peripheral immune activation and CNS leukocyte trafficking are therapeutic targets in immune-mediated CNS inflammatory disorders such as multiple sclerosis. The efficacy of immune-modulating and anti-trafficking therapies in controlling disease activity in multiple sclerosis and the animal model EAE validate such therapeutic strategies (Aharoni et al., 2000; Yednock et al., 1992).

We hypothesized that inhibiting cyclophilin A would ameliorate the severity of immune-mediated CNS inflammation through modulation of T helper response and/or CNS leukocyte trafficking. To test this hypothesis, we induced EAE in cyclophilin A knockout mice, and now report the results of studies assessing the course and neuroimmunology of EAE induced in cyclophilin A knockout mice.

2. Materials and methods

2.1 Reagents

All reagents were obtained from Fisher Scientific, unless otherwise specified. MOG_{35-55} peptide was obtained from Anaspec (Fremont, CA). Complete Freund's adjuvant (CFA) was prepared by adding M. Tuberculosis H37 RA (Difco, Detroit MI) to incomplete Freund's adjuvant (Difco). Pertussis toxin was obtained from List Biological Laboratories (Campbell, CA).

2.2 Animals

Cyclophilin A knockout mice on C57BL/6 background, obtained from the laboratory of Bradford C. Berk, were used to establish a local breeding colony. A heterozygous mating scheme was used to produce homozygous cyclophilin A knockout (i.e. *Ppia*−/−) and wild type (i.e. *Ppia*+/+) littermates. Mice were genotyped by PCR using the following primers: common forward primer - CAC CCT GGA GCA CCA CTG CCC ACC; wild type reverse primer - GCA GTT GTG ATT GAT CCA GGT CCG; cyclophilin A knockout reverse primer - CCT GAT CGA CAA GAC CGG CTT CC. Animals were housed in groups, on a

12 h light-dark cycle and fed ad libitum. All animal experiments were conducted under a protocol approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

2.3 EAE induction and treatment

EAE was actively induced in 6 to 10-week old female and male mice by subcutaneous injection of 200 μg MOG_{35-55} peptide in CFA and intraperitoneal injections of 300 ng pertussis toxin and scored daily as previously described (Huang et al., 2017). Clinical score rater was blinded to genotype. Mice were scored as follows: 0 – no overt signs of disease; 1 – limp tail or loss of righting reflex but not both; 2 – limp tail and loss of righting reflex; 3 – partial hind limb paralysis; 4 – complete hind limb paralysis; 5 – moribund state or death. Cyclophilin A knockout mice that developed blepharitis were excluded from analysis (Colgan et al., 2004).

2.4 Ex vivo *antigen-recall response*

Following euthanasia, spleens were removed from EAE-induced mice on days 14 to 15 post induction. Single cell suspensions of splenocytes were prepared by passing the spleen through a 70 μm strainer, then resuspending cells in ammonium-chloride-potassium buffer to lyse erythrocytes. Cells were washed and resuspended in RPMI supplemented with antibiotics and 10% fetal calf serum. Cells were plated into round-bottom 96-well plates at 1×10^6 per well and stimulated for 72 hours with MOG_{35-55} (20 μg/ml), phytohemagglutinin (2% v/v) or phosphate buffered saline (PBS). Supernatant was collected and stored frozen for later use in enzyme linked immunosorbent assay (ELISA). Splenocytes were fixed and permeabilized using a fixation/permeabilization kit (eBioscience) and used for intracellular antigen staining (Ki67 or Foxp3).

2.5 Fluorescence-activated cell sorting (FACS) analysis

Following euthanasia, animals underwent transcardiac perfusion with up to 50 ml of normal saline using a rate controlled pump. Thoracolumbar cords (from T2 to caudal end) were expelled out of the spinal column using hydraulic pressure manually applied through a 19-gauge needle and syringe filled with PBS. Cords were minced using a McIlwaine tissue chopper (Mickle Laboratory Eng. Co., UK), then enzymatically dissociated in RPMI media containing 2.5 mg/ml collagenase D (Roche Diagnostics, Indianapolis, IN) and DNasel (20 μg/ml, Sigma Aldrich) for 45 minutes at 37°C on a tube rotator. Cells were passed through a 70 μm strainer, washed in RPMI, resuspended in 30% isotonic Percoll (GE Healthcare) in PBS, then centrifuged at 500 × g for 10 min. Supernatant was removed. Cell pellets were washed and resuspended in RPMI. Cells were aliquoted into tubes and washed in FACS buffer (0.1% sodium azide and 2% fetal calf serum in PBS). Cells were incubated with Fc block (anti-mouse CD16/CD32 antibody, BD Biosciences) for 5 minutes prior to addition of fluorochrome-conjugated antibodies against CD3 (clone 17A2), CD4 (clone GK1.5), CD45 (clone 30-F11), CD19 (clone 1D3), CD8 (clone 53-6.7), CD11b (clone M1/70), CD11c (clone N418), Ly6C (clone HK1.4), Ly6G (clone 1A8-Ly6G) and NK1.1 (clone PK136). Following 30-minute incubation in the dark at 4°C, cells were washed in FACS buffer. Count beads (CountBright, Thermo Fisher) were added to each sample to allow absolute count determination.

For intracellular staining of Foxp3 and Ki67, fixed and permeabilized splenocytes obtained from *ex vivo* antigen-recall response assay were incubated with normal goat serum (2% in FACS buffer) to block non-specific binding. Antibodies against the following antigens were added for 30 minutes in the dark: CD4 (clone RM4-5), CD3 (clone 17A2), CD25 (clone 7D4), Foxp3 (clone FJK-16s) and Ki67 (clone B56). Cells were then washed in permeabilization buffer (eBioscience) then resuspended in FACS buffer.

FACS data was acquired on a flow cytometer (FACS Canto, BD Biosciences). Data were analyzed using FlowJo software (FlowJo, LLC).

2.6 Immunohistochemistry

Following euthanasia, animals underwent transcardiac perfusion with up to 50 ml of normal saline followed by perfusion with 100 ml of 4% (w/v) paraformaldehyde in PBS using a rate controlled pump. Following perfusion fixation, the entire CNS tissue was dissected out then cryoprotected in 30% sucrose in PBS for over 48 h. Spinal cords were further sectioned into 1 cm length sections measured from the cervicomedullary junction caudally under a dissecting microscope to approximate cervical, thoracic and lumbosacral cords, then cryopreserved at −80°C in Optimal Cutting Temperature compound. For CD3+ T cell immunohistochemistry, 20 μm thick serial sections were obtained through the entire lumbosacral cord in a ventral to dorsal direction for each animal, and every fourth section was processed for immunohistochemistry. Following rinses in Tris buffered saline (TBS), sections were blocked in blocking buffer comprised of 0.3% Triton X-100 and cold water fish skin gelatin (Sigma-Aldrich) in TBS for 15 minutes. Antibodies against CD3 (clone 500A2; BD Pharmingen) and CD31 (as a marker of endothelium; clone ER-MP12), were applied to the tissue sections for one hour at room temperature. The sections were then washed with TBS and blocked for 15 minutes in blocking buffer. Alexa Fluor conjugated secondary antibodies against respective primary antibodies were applied for one hour at room temperature. Sections were rinsed in TBS, then mounted with Vectashield (Vector Laboratories, Burlingame CA). Images from two non-overlapping fields of view per section were obtained on a Zeiss Axiolmager Z2 fluorescence microscope at 5 × (0.16 aperture) objective. ImageJ was used to quantify CD3 counts from single-channel CD3 immunolabeled images, utilizing the threshold, watershed and analyze particles function of ImageJ. Ten non-overlapping images (from 5 sections) were analyzed for each animal.

2.7 Statistical Analysis

For two group comparisons, the effect size, 95% confidence interval (CI) and test of statistical significance were assessed. Unpaired t-test was used for two group comparisons where normality of data could be assumed. Otherwise Mann-Whitney was used for two group comparisons. Statistical analysis was performed using GraphPad Prism software (San Diego, CA).

3. Results

3.1 Clinical course of EAE in the cyclophilin A knockout mice

Mice heterozygous for the cyclophilin A gene were mated to produce homozygous cyclophilin A knockout and wild type littermates. The prevalence of cyclophilin A knockout mice in the litter was 14%, or lower than expected by chance, and suggested a lower viability of cyclophilin A knockout mice compared to wild type, consistent with a previous report (Colgan et al., 2004). At 6 to 8 weeks of age, cyclophilin A knockout mice were significantly smaller on average compared to wild type littermates (12.2 g vs 16.8 g, unpaired t-test $p < 0.001$). Difference in size meant that blinding was ineffective for research personnel when assessing EAE clinical scores: post-hoc survey showed that a blinded research personnel could correctly guess the genotype for more than 80% of the mice.

EAE was actively induced in cyclophilin A knockout mice and wild type littermates. There was a trend towards lower incidence of EAE in cyclophilin A knockout mice compared to wild type littermates that did not reach statistical significance (76.9% vs. 100% respectively, Fisher's exact test $p = 0.0704$). There was no difference in mortality associated with EAE between cyclophilin A knockout mice and wild type littermates (26.7% vs. 19.4% respectively, Fisher's exact test $p = 0.56$). When all EAE-induced mice were analyzed, cyclophilin A knockout mice appeared to have less severe course of EAE (Figure 1A). However, among mice that developed EAE (i.e. excluding mice that did not manifest clinical signs of EAE), the clinical course including onset and peak severity were similar between cyclophilin A knockout and wild type littermates (Figure 1B).

3.2 Interferon-γ production in response to CNS antigen is impaired in cyclophilin A knockout mice

EAE-induced cyclophilin A knockout mice showed relative splenomegaly compared to EAE-induced WT littermates when analyzed as percent of total body weight (0.74% vs. 0.58%): difference in means of 0.17; 95% CI 0.023 to 0.31; unpaired t-test $p = 0.0241$. The relative splenomegaly in EAE-induced cyclophilin A knockout mice suggested that peripheral immune response may be altered in the cyclophilin A knockout mice. To detect changes in peripheral immune activation associated with EAE, *ex vivo* antigen recall response to MOG_{35-55} peptide was assessed in splenocytes from EAE-induced cyclophilin A knockout mice and wild type littermates. Interferon-γ release against MOG_{35-55} was significantly reduced in splenocytes from cyclophilin A knockout mice compared to wild type littermates: difference in means of −3560 pg/ml; 95% CI −5970 to −1149; unpaired t-test $p = 0.0059$ (Figure 2A), suggesting that T helper 1 (Th1) response is blunted during EAE in cyclophilin A knockout mice. Neither IL-4 levels (difference in means of 5.26 pg/ml; 95% CI −10.8 to 21.3; unpaired t-test $p = 0.503$) nor IL-17 levels (difference in means of 227.4 pg/ml; 95% CI −7.049 to 461; unpaired t-test = 0.0566) were substantially altered in response to *ex vivo* MOG_{35-55} stimulation (Figure 2A). There was no significant difference in the frequency of CD4+CD25+Foxp3+ regulatory T cells (Figure 2B). CD4+ T cell proliferation in response to MOG_{35-55}, assessed by Ki67 expression, was not significantly altered in cyclophilin A knockout mice (Figure 2C).

3.3 T cell infiltration into the CNS was reduced in cyclophilin A knockout mice

Cyclophilin A was shown to contribute to leukocyte chemotaxis in several inflammatory disorders. To determine whether or not the loss of cyclophilin A alters inflammatory cell infiltration into the CNS during EAE, we performed FACS analysis of thoracolumbar cords from EAE-induced mice to phenotypically enumerate CNS immune cell infiltration (Figure 3A). Total leukocyte (CD45+) counts were reduced in cyclophilin A knockout mice compared to wild type littermates: difference in means of −491300 cells/cord; 95% CI − 958548 to −24053; unpaired t-test p = 0.0406 (Figure 3B). Among leukocyte subsets, absolute counts of CNS infiltrating CD3+ T cells were reduced in cyclophilin A knockout mice: difference in means of −92778 cells/cord; 95% CI −148811 to −36744; unpaired t-test p = 0.0030 (Figure 3B). CD3+CD4+ T cells, in particular, were substantially reduced among CD3+ T cells in cyclophilin A knockout mice: difference in means of −36755 cells/cord; 95% CI −63345 to −10165; unpaired t-test p = 0.010 (Figure 3B). There were no significant differences in the other leukocyte subsets identified by FACS analysis (Figure 3B).

Immunohistochemical analysis of CNS infiltrating CD3+ T cells was performed to confirm the results obtained by FACS analysis using a different methodology. Analysis of lumbar cord CD3 immunohistochemistry (Figure 3C) showed significant reduction in mean CD3+ T cell counts in the lumbar cords of cyclophilin A knockout mice compared to wild type littermates during EAE: difference in means of −2280 cells/section; 95% CI −3430 to −944; Mann Whitney p = 0.0121 (Figure 3D).

3.4 Exploratory analysis of sex differences in EAE-induced cyclophilin A knockout mice

Post-hoc analyses were performed to detect possible sex differences in outcome. Incidence of EAE was 85.7% (6 out of 7) in female and 60% (3 out of 5) in male cyclophilin A knockout mice (Fisher's exact test p = 0.5227). The clinical course comparing cyclophilin A knockout mice and wild type littermates based on sex, excluding animals that did not develop EAE, is shown in Figure 4. Data from a small number (N = 3) of male cyclophilin A knockout mice were available for comparison. Male cyclophilin A knockout mice appear to have a more severe initial course followed by a later course similar to male wild type littermates. Interferon-γ production in response to *ex vivo* MOG re-stimulation was lower for both female and male cyclophilin A knockout mice compared to wild type littermates (Table). Leukocyte recruitment remained significantly lower in cyclophilin A knockout mice when only females were analyzed, but differences were no longer statistically significant when only males were analyzed (Table).

4. Discussion

We compared the course and neuroimmunology of EAE in cyclophilin A knockout mice and wild type littermates to test the hypothesis that loss of cyclophilin A would result in amelioration of EAE. We found that interferon-γ release and CNS T cell infiltration were reduced in cyclophilin A knockout mice compared to wild type littermates. There was a trend towards lower incidence of EAE, but the clinical severity of EAE did not differ substantially between cyclophilin A knockout and wild type littermates among animals that

manifested clinical signs of EAE, indicating, at best, a modest impact of these specific immunologic changes on the clinical course of EAE.

Cyclophilin A knockout mice showed altered peripheral immune activation to CNS antigen. We found that interferon-γ release in recall response to MOG peptide was substantially reduced in EAE-induced cyclophilin A knockout mice. IL-4 and IL-17 release were not significantly altered. These results suggest that whereas Th1 response during EAE was blunted in cyclophilin A knockout mice, the overall T helper lineage deviation associated with MOG-induced EAE appeared unchanged. It was previously shown that cyclophilin A modulates T cell receptor signaling by interaction with ITK, where the loss of cyclophilin A resulted in derepression of Th2 response resulting in increased IL-4 production (Brazin et al., 2002; Colgan et al., 2004). We did not find increased IL-4 production in EAE-induced cyclophilin A knockout mice, indicating that reduction in interferon-γ release is not explained by a shift in T helper lineage differentiation. MOG-induced EAE remained predominantly a Th1/Th17 response in cyclophilin A knockout mice. Further studies are needed to explain the mechanism whereby cyclophilin A influences interferon-γ production.

The role of cyclophilin A in CNS leukocyte recruitment has not previously been described. Our results showed that CNS leukocyte infiltration during EAE was reduced in cyclophilin A knockout mice. Leukocyte subset analysis indicated that CD3+ T cell infiltration into the CNS was reduced in cyclophilin A knockout mice, affecting CD3+CD4+ T cells, in particular. This finding that CNS T cell infiltration is reduced in cyclophilin A knockout mice adds to the well-described function of cyclophilin A as a leukocyte chemotactic factor. Previous reports showed that blocking cyclophilin A reduced infiltration of T cells, monocytes/macrophages, neutrophils and eosinophils in models of allergic lung and heart diseases (Arora et al., 2005; Balsley et al., 2010; Heinzmann et al., 2015; Iordanskaia et al., 2015). We showed that T cell infiltration into the CNS is reduced in cyclophilin A knockout mice by using two different but complementary methods. Whereas FACS analysis has the advantage of unbiased sampling from the entire cord at the cost of lost information regarding anatomical distribution, immunohistochemical analysis preserves information regarding anatomical distribution at the cost of more limited sampling. Both methods showed reduction in CD3+ T cell infiltration into the CNS in cyclophilin A knockout mice. Thus our results support a role for cyclophilin A in CNS recruitment of T cells during immune-mediated inflammation. Based on prior literature, we would expect that CD147 serves as the chemotactic receptor for cyclophilin A-mediated CNS T cell infiltration (Agrawal et al., 2011; Yurchenko et al., 2002).

The clinical course of EAE was similar in cyclophilin A knockout mice and wild type littermates. There are several possible explanations as to why the immunologic changes observed in cyclophilin A knockout mice did not correspond to a substantial change in the clinical course of EAE. One possible explanation is that the change in immune activation observed in cyclophilin A knockout mice is dispensable for the pathophysiology of EAE. Interferon-γ, reduced in cyclophilin A knockout mice, was previously shown to be dispensable for susceptibility to EAE based on studies in interferon-γ knockout mice (Ferber et al., 1996). Another possible explanation is that the reduction in CNS infiltrating CD3+ T cells was partial, and did not reach a threshold needed to impact EAE clinical

scores. A previous study showed that blocking CD147, which functions as chemotactic receptor for cyclophilin A, ameliorates the clinical course of EAE (Agrawal et al., 2011). It is interesting to note that whereas blocking CD147 resulted in reduced numbers of CNS infiltrating CD4+ T cells and macrophage/microglia (Agrawal et al., 2011), we found that cyclophilin A knockout mice had reduced numbers of CNS CD4+ T cells without changes in macrophage or microglia counts. CD147 has been shown to interact with other chemotactic factors such as E-selectins (Kato et al., 2009), and therefore it remains possible that the loss of cyclophilin A only partially recapitulates the effect of blocking CD147.

Post-hoc analysis to detect possible sex differences in outcome indicated that the reduction in interferon-γ production were observed in both female and male cyclophilin A knockout mice. Leukocyte recruitment remained significantly lower in female cyclophilin A knockout mice compared to female wild type. CNS-infiltrating T cell counts were lower, but differences were not statistically significant, in male cyclophilin A knockout mice compared to male wild type. One possible explanation is that the analysis is underpowered for male cyclophilin A knockout mice. Another possible explanation is the presence of true sex difference in cyclophilin A knockout mice with respect to CNS leukocyte recruitment. Further work is needed to clarify this. Clinical course of EAE in female cyclophilin A knockout mice appeared similar to female wild type, thus sex differences does not appear to explain the lack of correspondence between changes in the immune response and clinical course of EAE in cyclophilin A knockout mice.

This study has several limitations. This study was powered to detect a difference in mean EAE clinical score of 1.0. The study was not powered to detect smaller differences in mean clinical scores or to detect small differences in incidence of EAE. A more sensitive clinical outcome measure such as the rotarod (van den Berg et al., 2016) may better detect differences in clinical outcome in cyclophilin A knockout mice. The study was not powered to detect sex differences in outcome. We did not assess peripheral immune activation and CNS leukocyte infiltration in mice that did not manifest clinical signs of EAE. Furthermore, we did not assess the cytokine profile of CNS-infiltrating T cells in this study and therefore have not excluded the possibility that CNS-infiltrating T cell cytokine profile may differ from that observed in the periphery. It remains possible that the cytokine profile of CNS-infiltrating T cells may better explain the clinical outcome.

5. Conclusion

Although the loss of cyclophilin A was associated with reduced interferon-γ production and reduced CNS T cell infiltration during EAE, clinical course did not differ substantially in cyclophilin A knockout mice compared to wild type littermates. We predict that targeting cyclophilin A would have, at best, a modest impact on the clinical course of immune-mediated CNS inflammatory disorders such as multiple sclerosis.

Acknowledgements

This study was funded by the National Institute of Neurological Disorders and Stroke (R03 NS098123). Microscopy and flow cytometry were performed at the VCU – Dept. of Anatomy & Neurobiology Microscopy

Facility and at the VCU Massey Cancer Center Flow Cytometry Shared Resource, supported, in part, with funding from the NIH-NCI Cancer Center Support Grant P30 CA016059.

References

Agrawal SM, Silva C, Tourtellotte WW, Yong VW, 2011 EMMPRIN: a novel regulator of leukocyte transmigration into the CNS in multiple sclerosis and experimental autoimmune encephalomyelitis. J Neurosci 31, 669–77. 10.1523/JNEUROSCI.3659-10.2011 [PubMed: 21228176]

Aharoni R, Teitelbaum D, Leitner O, Meshorer A, Sela M, Arnon R, 2000 Specific Th2 cells accumulate in the central nervous system of mice protected against experimental autoimmune encephalomyelitis by copolymer 1. Proc Natl Acad Sci U S A 97, 11472–7. 10.1073/pnas.97.21.11472 [PubMed: 11027347]

Arora K, Gwinn WM, Bower MA, Watson A, Okwumabua I, MacDonald HR, Bukrinsky MI, Constant SL, 2005 Extracellular cyclophilins contribute to the regulation of inflammatory responses. J Immunol 175, 517–22. [PubMed: 15972687]

Balsley MA, Malesevic M, Stemmy EJ, Gigley J, Jurjus RA, Herzog D, Bukrinsky MI, Fischer G, Constant SL, 2010 A cell-impermeable cyclosporine A derivative reduces pathology in a mouse model of allergic lung inflammation. J Immunol 185, 7663–70. 10.4049/jimmunol.1001707 [PubMed: 21057089]

Brazin KN, Mallis RJ, Fulton DB, Andreotti AH, 2002 Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A. Proc Natl Acad Sci U S A 99, 1899–904. 10.1073/pnas.042529199 [PubMed: 11830645]

Christofferson DE, Yuan J, 2010 Cyclophilin A release as a biomarker of necrotic cell death. Cell Death Differ 17, 1942–3. 10.1038/cdd.2010.123 [PubMed: 20930851]

Colgan J, Asmal M, Neagu M, Yu B, Schneidkraut J, Lee Y, Sokolskaja E, Andreotti A, Luban J, 2004 Cyclophilin A regulates TCR signal strength in CD4+ T cells via a proline-directed conformational switch in Itk. Immunity 21, 189–201. 10.1016/j.immuni.2004.07.005 [PubMed: 15308100]

Damsker JM, Bukrinsky MI, Constant SL, 2007 Preferential chemotaxis of activated human CD4+ T cells by extracellular cyclophilin A. J Leukoc Biol 82, 613–8. 10.1189/jlb.0506317 [PubMed: 17540735]

Dear JW, Simpson KJ, Nicolai MP, Catterson JH, Street J, Huizinga T, Craig DG, Dhaliwal K, Webb S, Bateman DN, Webb DJ, 2011 Cyclophilin A is a damage-associated molecular pattern molecule that mediates acetaminophen-induced liver injury. J Immunol 187, 3347–52. 10.4049/jimmunol.1100165 [PubMed: 21824865]

Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, Dalton D, Fathman CG, 1996 Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J Immunol 156, 5–7. [PubMed: 8598493]

Heinzmann D, Bangert A, Muller AM, von Ungern-Sternberg SN, Emschermann F, Schonberger T, Chatterjee M, Mack AF, Klingel K, Kandolf R, Malesevic M, Borst O, Gawaz M, Langer HF, Katus H, Fischer G, May AE, Kaya Z, Seizer P, 2015 The Novel Extracellular Cyclophilin A (CyPA) - Inhibitor MM284 Reduces Myocardial Inflammation and Remodeling in a Mouse Model of Troponin I -Induced Myocarditis. PLoS One 10, e0124606 10.1371/journal.pone.0124606 [PubMed: 25894208]

Huang ZL, Pandya D, Banta DK, Ansari MS, Oh U, 2017 Cyclophilin inhibitor NIM811 ameliorates experimental allergic encephalomyelitis. J. Neuroimmunol 311, 40–48. 10.1016/j.jneuroim.2017.07.016 [PubMed: 28789840]

Iordanskaia T, Malesevic M, Fischer G, Pushkarsky T, Bukrinsky M, Nadler E, 2015 Targeting extracellular cyclophilins ameliorates disease progression in experimental biliary atresia. Mol Med. 10.2119/molmed.2015.00076

Kato N, Yuzawa Y, Kosugi T, Hobo A, Sato W, Miwa Y, Sakamoto K, Matsuo S, Kadomatsu K, 2009 The E-selectin ligand basigin/CD147 is responsible for neutrophil recruitment in renal ischemia/reperfusion. J Am Soc Nephrol 20, 1565–76. 10.1681/ASN.2008090957 [PubMed: 19443639]

Sherry B, Yarlett N, Strupp A, Cerami A, 1992 Identification of cyclophilin as a proinflammatory secretory product of lipopolysaccharide-activated macrophages. Proc Natl Acad Sci U S A 89, 3511–5. [PubMed: 1565646]

Suzuki J, Jin ZG, Meoli DF, Matoba T, Berk BC, 2006 Cyclophilin A is secreted by a vesicular pathway in vascular smooth muscle cells. Circ Res 98, 811–7. 10.1161/01.RES.0000216405.85080.a6 [PubMed: 16527992]

Takahashi N, Hayano T, Suzuki M, 1989 Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. Nature 337, 473–5. 10.1038/337473a0 [PubMed: 2644542]

van den Berg R, Laman JD, van Meurs M, Hintzen RQ, Hoogenraad CC, 2016 Rotarod motor performance and advanced spinal cord lesion image analysis refine assessment of neurodegeneration in experimental autoimmune encephalomyelitis. J. Neurosci. Methods 262, 66–76. 10.1016/j.jneumeth.2016.01.013 [PubMed: 26784021]

Xu Q, Leiva MC, Fischkoff SA, Handschumacher RE, Lyttle CR, 1992 Leukocyte chemotactic activity of cyclophilin. J Biol Chem 267, 11968–71. [PubMed: 1601866]

Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N, 1992 Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 356, 63–6. 10.1038/356063a0 [PubMed: 1538783]

Yurchenko V, Zybarth G, O'Connor M, Dai WW, Franchin G, Hao T, Guo H, Hung HC, Toole B, Gallay P, Sherry B, Bukrinsky M, 2002 Active site residues of cyclophilin A are crucial for its signaling activity via CD147. J Biol Chem 277, 22959–65. 10.1074/jbc.M201593200 [PubMed: 11943775]

Highlights

- Interferon-gamma release is reduced in cyclophilin A knockout mice in response to myelin oligodendrocyte protein (MOG) peptide
- CNS T cell infiltration is reduced in EAE-induced cyclophilin A knockout mice
- There was a trend towards lower incidence of EAE in cyclophilin A knockout mice, but clinical course was similar to wild type

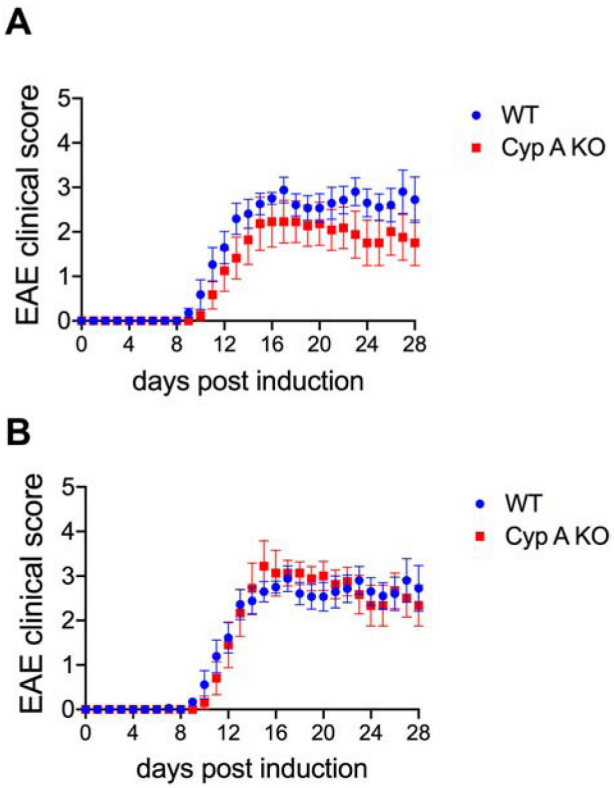

Figure 1. Clinical course of experimental allergic encephalomyelitis (EAE) in the cyclophilin A knockout (Cyp A KO) mice.

Cyp A KO mice and wild type (WT) littermates were actively induced to undergo EAE and scored daily. A) Shown are mean +/− S.E.M. of daily clinical scores for all Cyp A KO mice (N = 12: 7 females, 5 males) and WT littermates (N = 17: 9 females, 8 males). B) Mice that did not manifest clinical signs of EAE were excluded from analysis. Shown are mean +/− S.E.M. of daily clinical scores for Cyp A KO mice (N = 9; 6 females, 3 males) and WT littermates (N = 17: 9 females, 8 males). Data pooled from 5 independent experiments.

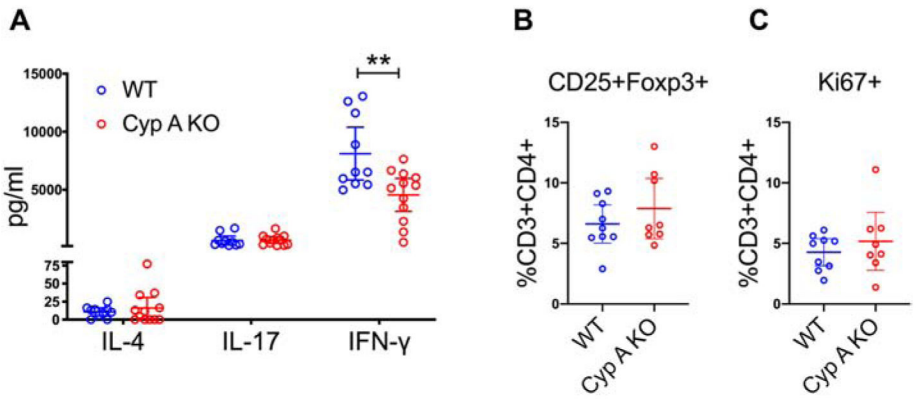

Figure 2. Interferon-γ production in response to CNS antigen is impaired in cyclophilin A knockout mice.

Splenocytes from EAE-induced cyclophilin A knockout mice (Cyp A KO) and wild type (WT) littermates were obtained on days 14 to 15 post induction to assess peripheral immune activation. Antigen recall response was assessed by *ex vivo* stimulation of splenocytes with MOG_{35-55} peptide. Cytokine release was assessed by ELISA at 72 h. Splenocytes were assessed for the frequency of Foxp3+ regulatory T cells and proliferating (Ki67+) CD4+ T cells by FACS analysis at 72 h. A) Shown are mean (+/− 95% confidence interval) for the indicated cytokines released by Cyp A KO or WT splenocytes in response to MOG_{35-55} stimulation. **, $p < 0.01$, otherwise $p \geq 0.05$ B) Mean (+/− 95% confidence interval) frequencies of CD3+CD4+CD25+Foxp3+ regulatory T cells. $p \geq 0.05$. C) Mean (+/− 95% confidence interval) frequencies of CD3+CD4+Ki67+ T cells. $p \geq 0.05$.

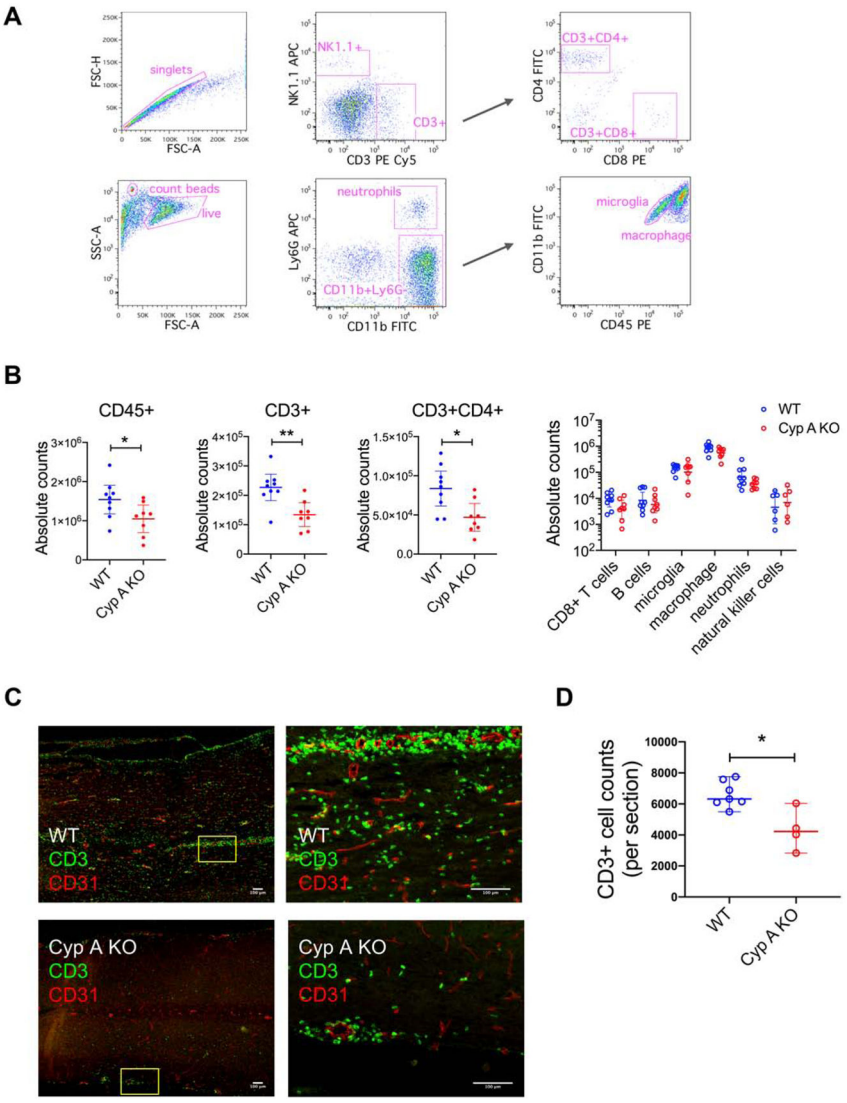

Figure 3. T cell infiltration into the CNS was reduced in cyclophilin A knockout mice.

Single-cell suspensions were prepared from thoracolumbar cords of cyclophilin A knockout (Cyp A KO) mice and wild type (WT) littermates on days 14 to 15 post induction of EAE. CNS infiltrating leukocytes were phenotypically enumerated by FACS analysis. In a separate analysis, CNS infiltrating CD3+ T cells were analyzed by immunohistochemistry of lumbar cords from Cyp A KO and WT littermates. A) Representative FACS analysis of CNS inflammatory infiltrates. B) Shown are mean absolute counts (+/− 95% confidence interval) per cord for the indicated leukocyte subsets. *, $p < 0.05$. **, $p < 0.01$, otherwise $p \geq 0.05$ C) Representative images of lumbar cord CD3 (green) immunohistochemistry on the left with high magnification images of inset shown on the right. CD31 (red) immunolabeling identifies endothelium. Scale bar =100 μm. D) Analysis of immunohistochemistry data showing mean CD3+ cell counts (+/− 95% confidence interval). * $p < 0.05$.

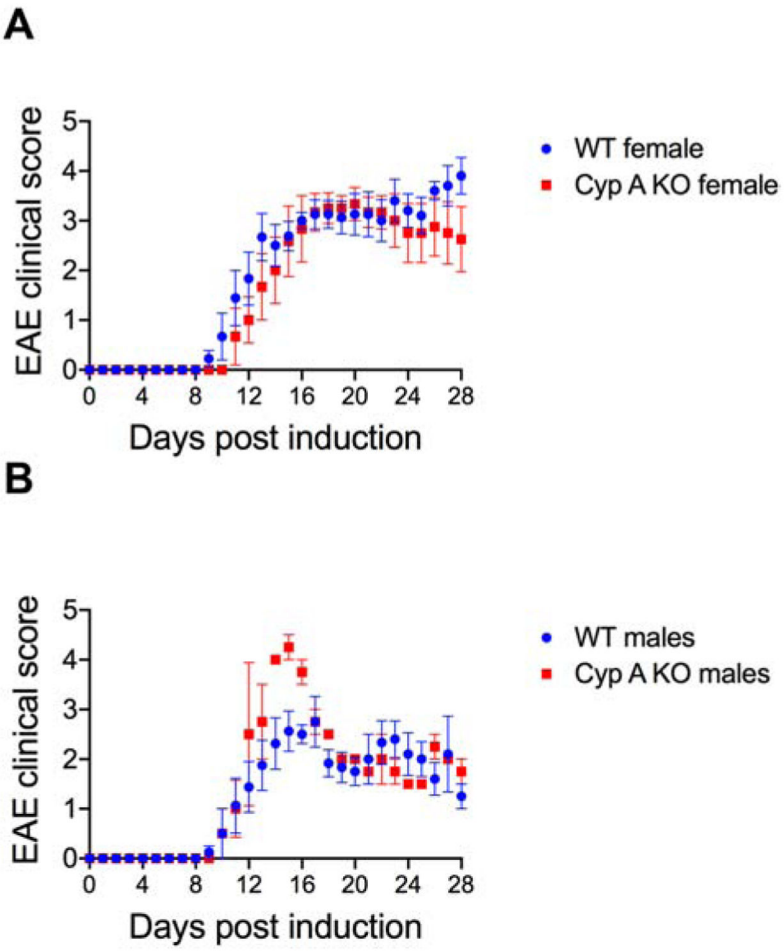

Figure 4. Sex differences in the clinical course of experimental allergic encephalomyelitis (EAE) in cyclophilin A knockout (Cyp A KO) mice and wild type (WT) littermates.

A) Shown are mean +/− S.E.M. of daily clinical scores for female mice (N = 9 WT and 6 Cyp A KO). B) Mean +/− S.E.M. of daily clinical scores for male mice (N = 8 WT and 3 Cyp A KO). Data pooled from 5 independent experiments. Mice that did not manifest clinical signs of EAE were excluded from analysis.

Table.

Exploratory analysis of sex differences in EAE-induced cyclophilin A knockout mice

	N (WT, Cyp A KO)	Effect size (difference in means)	95% CI	T-test p-value
Interferon-γ(female)	4, 6	−2825 pg/ml	−5134 to −516	0.0224
Interferon-γ(male)	6, 6	−3499 pg/ml	−65356 to −461	0.0281
Absolute CD45+ counts (female)	5, 5	−555647 cells/cord	−995439 to −115858	0.0195
Absolute CD45+ counts (male)	4, 3	−419694 cells/cord	−1732978 to 893590	0.449
Absolute CD3+ counts (female)	5, 5	−66928 cells/cord	−11342 to −22514	0.0084
Absolute CD3+ counts (male)	4, 3	−19193 cells/cord	−80844 to 42458	0.460
Absolute CD3+CD4+ counts (female)	5, 5	−49252 cells/cord	−81481 to −17023	0.0078
Absolute CD3+CD4+ counts (male)	4, 3	−20467 cells/cored	−80083 to 39148	0.418