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Supplemental Information

Type 2 Innate Lymphoid Cells Induce CNS Demyelination in an HSV-IL-2 Mouse Model of Multiple Sclerosis

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1 STAR★Methods

2 Key Resources Table

REAGENT or RESOURCE	SOURCR	IDENTIFIER
Antibodies		
Biotin-conjugated anti-mouse CD3e (145-2C11)	BioLegend	Cat#100304
Biotin-conjugated anti-mouse CD5 (53-7.3)	BioLegend	Cat#100604
Biotin-conjugated anti-mouse CD45R (RA3-6B2)	BioLegend	Cat#103204
Biotin-conjugated anti-mouse Gr-1 (RB6-8C5)	BioLegend	Cat#108404
Biotin-conjugated anti-mouse CD11c (N418)	BioLegend	Cat#117304
Biotin-conjugated anti-mouse CD11b (M1/70)	BioLegend	Cat#101204
Biotin-conjugated anti-mouse Ter119 (TER-119)	BioLegend	Cat#116204
Biotin-conjugated anti-mouse FcεRIa (MAR-1)	BioLegend	Cat#134304
Biotin-conjugated anti-mouse TCR-gd (eBioGL3)	ThermoFisher Scientific	Cat#13-5711-82
PECy7-conjugated anti-mouse CD127 (A7R34)	BioLegend	Cat#135014
APCCy7-conjugated anti-mouse CD45 (30-F11)	BioLegend	Cat#103116

PE-conjugated anti-mouse ST2 (DIH9)	BioLegend	Cat#145303
anti-mouse CD16/32 (93)	BioLegend	Cat#101302
Alexa Fluor 488-conjugated anti-mouse IL-2 (JES6-5H4)	BioLegend	Cat#503813
Bacterial and Virus Strains		
LAT2903	Homayon Ghiasi, Cedars-Sinai Medical Center (Perng et al., 1994)	N/A
HSV-IL-2	Homayon Ghiasi, Cedars-Sinai Medical Center (Ghiasi et al., 2002)	N/A
Chemicals, Peptides, and Recombinant Proteins		
Minimum Essential Medium (MEM)	Corning	Cat#10-010-CV
Fetal Bovine Serum (FBS)	Omega Biosciences	Cat#FB-02
Mouse recombinant IL-33	BioLegend	Cat#580508
Streptavidin-FITC	BioLegend	Cat#405202
Prolong Gold Antifade Mountant with DAPI	ThermoFisher Scientific	Cat#P36931
O.C.T. compound	Sakura Finetek	Cat#4583
Trizol reagent	ThermoFisher Scientific	Cat#15596026
Critical Commercial Assays		
High-Capacity cDNA Reverse Transcription Kit	ThermoFisher Scientific	Cat#4368813

Experimental Models: Cell Lines		
Rabbit skin (RS) cells	Homayon Ghiasi, Cedars-Sinai Medical Center (Perng et al., 1994)	N/A
Experimental Models: Organisms/Strains		
Mouse: C57BL/6	Jackson Laboratory	Stock#000664
Mouse: IL-2rα$^{-/-}$	Jackson Laboratory	Stock#002952
Mouse: IL-2rβ$^{-/-}$	Jackson Laboratory	Stock#002816
Mouse: IL-2rγ$^{-/-}$	Jackson Laboratory	Stock#003174
Mouse: ILC1$^{-/-}$	Jackson Laboratory	Stock#004648
Mouse: ILC2$^{-/-}$	Dr. ANJ Mackenzie (MRC Laboratory of Molecular Biology, United Kingdom)	N/A
Mouse: ILC3-/-	Jackson Laboratory	Stock#007571
Oligonucleotides		
TaqMan Gene Expression Assay, see Materials and methods	ThermoFisher Scientific	Cat#4331182
Software and Algorithms		
Graphpad Prism (ver. 4)	Graphpad	N/A
Connect Data Analysis Apps	ThermoFisher Scientific	https://www.thermofisher.com/us/en/

		home/digital-science/thermo-fisher-connect/all-analysis-modules.html

Experimental Model and Subject Details

Cells and Virus. Rabbit skin (RS) cells were generated in our laboratory, prepared, grown in minimal essential medium (MEM) media plus 5% FBS and used as described previously (Perng et al., 1994). Plaque-purified HSV-1 recombinant virus expressing IL-2 (HSV-IL-2) and parental virus for HSV-IL-2 (LAT2903) were grown in RS cell monolayers in MEM containing 5% fetal calf serum, as we described previously (Ghiasi et al., 2002a; Ghiasi et al., 2001, 2002b).

Mice. Inbred IL-2r$\alpha^{-/-}$, IL-2r$\beta^{-/-}$, IL-2r$\gamma^{-/-}$, ILC1$^{-/-}$, and ILC3$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME), ILC2$^{-/-}$ mice were a gift from Andrew McKenzie (MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, United Kingdom) and described previously (Halim et al., 2018; Rafei-Shamsabadi et al., 2018). All mice have a B6 background and were bred in-house. WT C57BL/6 mice were used as a control. Only female mice (6 to 8-wk-old) were used in the study due to more resistance of male mice to CNS demyelination (Zandian et al., 2009). All animal procedures were performed in strict accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and the NIH *Guide for the Care and Use of Laboratory Animals* (ISBN 0-309-05377-3). The animal research protocol was approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center (Protocol #6134).

Ocular Infection. Female mice were infected ocularly with 2 X 10^5 PFU per eye of HSV-IL-2 or parental virus, in 2μl of tissue culture media as an eye drop without corneal scarification as we have described previously (Hirose et al., 2019; Mott et al., 2015). The presence of infectious virus in the eye of ocularly infected mice on days 1-5 post infection and the presence of viral DNA on day 14 post infection were monitored by PCR. Infectious virus was detected in all infected mice (not shown).

Analysis of demyelination using Luxol Fast Blue (LFB) staining. The presence or absence of demyelination in ON, SC, and brains of infected mice was evaluated using LFB staining of formalin-fixed sections of ON, SC, and brain as we described previously (Osorio et al., 2005). Every 4th section of ON, SC, and brain was stained with LFB. The number of plaques, size of plaques, and shape of plaques on multiple fields were evaluated by investigators who were blinded to the treatment groups using serial sections of CNS tissues. The amount of myelin loss in the stained sections of brains, SCs and ONs was measured using the NIH Image J software analysis system. The areas of demyelination (clear-white) to normal tissue (blue) were quantified using 150 random sections from the brain and SCs or 30 sections from ONs of each animal. Demyelination in each section was confirmed by monitoring adjacent sections. The percentage of myelin loss was calculated by dividing the lesion size into the total area for each section.

Isolation of type 2 ILCs. C57BL/6 mice were intraperitoneally treated with recombinant mouse (rm)IL-33 (IP; 1µg/mouse, BioLegend, San Diego, CA) for three days. On the fourth day, BM cells were collected from the femur and tibia and resuspended in phosphate buffered saline (PBS) solution containing 0.5% bovine serum albumin and 2 mM ethylenediaminetetraacetic acid (EDTA). BM ILC2s were stained based on the lack of expression of classical lineage markers (CD3e, CD5, CD45R, Gr-1, CD11c, CD11b, Ter119, TCRγδ, and FCεRI) and positive expression of CD45, ST2, and CD127 as previously described (Hirose et al., 2019; Hurrell et al., 2019; Rigas et al., 2017). The following mouse antibodies were used: biotinylated anti-mouse lineage CD3e (145-2C11), CD5 (53-7.3), CD45R (RA3-3B2), Gr-1 (RB6-8C5), CD11c (N418), CD11b (M1/70), Ter119 (TER-119), FcεRIa (MAR-1) (BioLegend) and TCR-gad (eBioGL3) (eBioscience). Streptavidin-FITC, PE-Cy7 anti-mouse CD127 (A7R34), APCCy7 anti-mouse CD45 (30-F11) were purchased from BioLegend. ILC2s were FACS purified using BD FACS ARIA III (BD Biosciences, San Jose, CA) with purity of >95%.

Immunostaining of ILC2. 5 X 10^4 ILC2 cells per tube were seeded in 5ml polystyrene round-bottom tube (Corning, Corning, NY) and infected with 10 PFU/cell of HSV-IL-2, parental virus or mock infected for 24 h. The cells were washed with staining buffer (cold PBS supplemented with 2% fetal calf serum and 0.05% sodium azide), and preincubated with anti-mouse CD16/32 (BioLegend) for 15 min., then incubated with anti-mouse IL-2 antibody conjugated with Alexa Fluor 488 (BioLegend) for 30 min.,

then washed once with staining buffer. Cells were fixed with 4 % paraformaldehyde in PBS for 10 min. at room temperature and then washed with staining buffer. Cells were mounted onto slide glass with Prolong Gold Antifade Mountant (ThermoFisher Scientific, Waltham, MA). The fluorophores were imaged by confocal microscopy using a Leica SP5-X confocal microscope, image acquisition and data analysis system (Leica Microsystems, Buffalo Grove, IL).

Adoptive transfer of ILC2s. To confirm the effect of ILC2s on CNS demyelination, ILC2s were isolated from bone-marrow of WT mice as described above and previously (Hirose et al., 2019). Each recipient ILC2$^{-/-}$ mouse was injected intravenously (IV) once with 1 X 10^5 ILC2s in MEM (100 μl). Mice were then infected ocularly with HSV-IL-2 virus two wk after transfer of the ILC2s.

RNA Extraction, cDNA Synthesis and TaqMan RT-PCR. Brains were collected from naive mice and mice that survived ocular infection on day 14 PI and individual brains were embedded in O.C.T. compound (Sakura Finetek, Tokyo, Japan), quickly frozen with dry ice, and stored at -80°C until sectioning. Brains were sectioned with Microm HM550 cryostat microtome (ThermoFisher) at 9 um thickness, -18ºC and 20 to 40 sections were collected in a 2ml sample tube. 0.5 ml Trizol reagent (ThermoFisher) was added and samples were stored at -80ºC until processing. Tissue processing, total RNA extraction, and RNA yield were performed as we have described previously (Mott et al., 2007a; Mott et al., 2007b). Following RNA extraction, 1000 ng of total RNA was reverse-transcribed using random hexamer primers and recombinant Molony Murine Leukemia Virus Reverse Transcriptase from the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's recommendations. The levels of various RNAs were evaluated using commercially available TaqMan Gene Expression Assays (Thermo Fisher Scientific) with optimized primer and probe concentrations. Primer probe sets consisted of two unlabeled PCR primers and the FAM™ dye-labeled TaqMan MGB probe formulated into a single mixture. All cellular amplicons also included an intron-exon junction to eliminate signal from genomic DNA contamination. The assays used in this study were as follows: 1) PD-1 (programmed death 1) ABI assay I.D. Mm00435532_m1 – Amplicon size 65 bp; 2) GM-CSF (Csf2 – colony stimulating factor 2 (granulocyte-macrophage)) ABI Mm01290062_m1 – Amplicon size 125 bp; 3) IL-5 (interleukin 5) ABI Mm00439646_m1 – Amplicon size 62 bp; 4) IL-6 (interleukin 6)

ABI Mm00446190_m1 – Amplicon size 78 bp; 5) IFNgr1 (interferon gamma receptor 1) Mm00599890_m1 – Amplicon size 85 bp; 6) RANTES (CCL5 – chemokine (C-C motif) ligand 5) ABI Mm01302427_m1 - Amplicon size 103 bp; 7) CXCL10 (chemokine (C-X-C motif) ligand 10) ABI Mm00445235_m1 – Amplicon size 59 bp; 8) 4-1BB (Tnfrsf9 – tumor necrosis factor receptor superfamily, member 9) ABI Mm00441899_m1 – Amplicon size 71 bp; 9) Hif1a (hypoxia inducible factor 1, alpha subunit) ABI Mm00468869_m1 – Amplicon size 75 bp; 10) Csf2rb (colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)) ABI Mm00655745_m1 – Amplicon size 125 bp; 11) TIM3 (Havcr2 – hepatitis A virus cellular receptor 2) ABI Mm00454540_m1 – Amplicon size 98 bp; 12) CTLA4 (cytotoxic T-lymphocyte-associated protein 4) ABI Mm00486849_m1 – Amplicon size 71 bp and 13) GAPDH used for normalization of transcripts, ABI Mm999999.15_G1 – Amplicon size 107 bp.

Quantitative real-time RT-PCR (qRT-PCR) was performed using QuantStudio 5 System (Thermo Fisher Scientific, Waltham, MA) in 384-well plates as described previously (Mott et al., 2007a; Mott et al., 2007b). The threshold cycle (CT) values, which represent the PCR cycles at which there is a noticeable increase in the reporter fluorescence above baseline, were determined using Applied Biosystems qPCR software (Thermo Fisher Scientific).

Statistical analyses. Student's t test and ANOVA were performed using the computer program Prism (GraphPad, San Diego, CA). Results were considered statistically significant when the "P" value was <0.05.