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Supplementary Information for

Lymphocytic Choriomeningitis Virus Cl 13 Infection Results in Either Persistence or Acute Death: Role for IFN-1, CTL, and Host Genetics

Michael B.A. Oldstone[a1], Brian C. Ware[a], Lucy E. Horton[a], Megan J. Welch[a], Roberto Aiolfi[b], Alessandro Zarpellon[b], Zaverio M. Ruggeri[b], and Brian M. Sullivan[a]

Michael B.A. Oldstone
Email: mbaobo@scripps.edu

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Materials and Methods

Mice and Viruses. C57Bl/6, BALB, SWR/J, NZB, FVB/N, A/J, PL/J, SJL/J, C3H/HeJ, 129S1Sv/lmJ, NOD/LtJ, NZO/H1Lt, CAST/EiJ, PWK/PhJ, and WSB/EiJ mice (6-8 weeks of age) were obtained from the rodent breeding colony at The Scripps Research Institute (TSRI) or Jackson Laboratory, Bar Harbor, Maine. All mice were maintained in pathogen-free conditions and handling conformed to the requirements of the NIH, TSRI Institutional Animal Care and Use Committee, and AAALAC. LCMV Cl 13 and LCMV ARM 53b were grown, stored, and quantified according to previously published methods (1-4). Mice were infected by intravenous injection of 2×10^6 plaque forming units (PFU) of virus. Both male and female mice were used and were equally susceptible to Cl 13 infection. For quantification of viremia, blood was drawn from the retro-orbital sinus under isoflurane anesthesia, serum or plasma obtained and viral loads quantitated by measurement of PFU on Vero E6 cells (2, 3, 5). Tissues (spleen, lung, liver, kidney, heart, and brain) were harvested from euthanized mice and frozen at -80°C. At time of infectious assay, tissues were thawed, weighed, and homogenized in sterile media (5% FCS) to 10% w/v, clarified by low-speed centrifugation and resultant supernatant utilized in plaque assays (3, 5).

Antibody Reagents and Treatments. To abort IFN-1 signaling mice were treated intraperitoneally (i.p.) with monoclonal antibody to interferon (IFN)-1 receptor (IFNAR) using antibody MAR1-5A3, IFN-1α with antibody clone TIF-3C5 that blocks only α-1, 4, 5, 11, and 13 or IFN-1β with clone HDβ-4A7 as described (6, 7). For deletion of CD8+ T cells we used monoclonal antibody YTS69 and for depletion of CD4+ T cells GK1.5 monoclonal antibody as described (4, 8). Efficiency of CD8+ and CD4+ T cell depletion was >98% as assayed by FACS analysis of blood and spleen cells on day 4. VL4 antibody

to LCMV nucleoprotein was purchased from BioXCell and used as reported (7). CD31 antibody was purchased from Biolegend Clone:390 and used as reported (8). Reagents and procedures used to detect PD-1, PD-L1, Lag3 at DCs have been reported (9, 10).

ELISA. Reagents to measure alpha and beta IFN-1 and their analysis was performed as described (6, 7).

Pulmonary Endothelial Studies. Mice were perfused with 10ml PBS by cardiac puncture, lungs harvested, diced and suspended in 1ml dissociation buffer (1mg/ml Collagenase D, 100ug/ml Dnase I) for 30 minutes at 37°C. Tissue was processed through 100um filters into single cell suspension. 10ml of RPMI was passed through the filters, material collected, centrifuged at 1500RPM 4°C, decanted, and collected supernatant suspended in 1ml of RBC lysis buffer (150mM NH4Cl, 10mM KHCO3, 100mM EDTA). After 1 minute, 10ml of cold RPMI buffer was added, cells spun at 1500 RPM at 4°C, decanted and resuspended in 1ml of FACS buffer (2% FBS in 1x PBS). Cells were plated at $1x10^6$ cells/well in a 96-well v-bottom plate. Antibodies against CD31-APC (Clone:390, Biolegend, California), CD45-BV421 (Clone: 30F11, Biolegend), CD19-PerCP-Cy5.5 (Clone 6D5, Biolegend), alpha-dystroglycan (α-DG) antibody (Clone IIH6, a gift from Kevin Campbell, University of Iowa School of Medicine) were diluted 1:200 and added to wells for 1hr at 4°C, cells washed and followed by anti-mouse IgM-FITC (Jackson ImmunoResearch, Pennsylvania) at 1:300 dilution for 30 min at 4°C. Cells were fixed with 4% Paraformaldehyde and gated on CD45-, CD19-, CD31+, α-DG+ on an LSR-II (Beckton Dickinson). To perform LCMV-antigen staining, all surface staining for pulmonary endothelial cells was done as above without α-DG antibody. The cells were fixed, permeabilized using Cytofix/Cytoperm kit (BD biosciences) and stained with anti-

LCMV NP-FITC (Clone: VL-4, BioXCell, New Hampshire) at 1:400 dilution for 1hr at 4°C. Samples were gated on CD45-, CD19-, CD31+, anti-LCMV NP+ on LSR-II (Beckton Dickinson).

Vascular Permeability. Infected and control mice at day 5.5 to 6 post-infection received 200 µl of Evans Blue dye (0.5% in PBS). After 20 min., mice were lethally anesthetized and perfused by intracardiac injection of 10 mL of PBS. Lungs were harvested, photographed and Evans Blue in the lung extracted and quantified (8).

Tissue Histology and Cell Isolation. Spleen, lungs, liver, kidneys, heart, and brain were harvested from infected and control mice placed in PBS buffered formalin, blocked in paraffin, 10 µm sections cut and stained with hematoxylin and eosin. Lungs harvested from PBS-perfused mice were initially diced into small pieces for analysis.

FACS. To detect expression of α-DG on or viral antigen expression in pulmonary endothelial cells or CD8 T cells in the lung we followed procedures of Baccala and Sullivan (4, 8). Isolation and identification of DCs, use of antibody to PD-1 and PD-L1, were outlined in our publications (3, 6, 7, 9, 11).

In Vivo CTL. We used a modification of the Liu and Whitton method (12). Briefly, NZO mice were infected with LCMV CL13 or ARM $2x10^6$ PFU i.v. Five days later mice were injected i.v. with 180ug of BFA in PBS. After 6hr, mice were perfused by cardiac puncture with 10ml PBS. Lungs were harvested in cold RPMI, chopped into small chunks and suspended in 500ul of dissociation buffer (1mg/ml Collagenase D, 100ug/ml Dnase I) on ice for 30 minutes. Tissue was processed into single cell suspension by pushing through 100um filters. Filter was washed with 10ml of RPMI, suspension collected and centrifuged at 1500RPM at 4°C. Resultant supernatant was decanted, resuspended in 1ml of RBC lysis

buffer (150mM NH4Cl, 10mM KHCO3, 100mM EDTA) for 1 minute. Thereafter 10ml of cold RPMI buffer was added, cells spun at 1500 RPM at 4°C, supernatant decanted and cells resuspended in 1ml of FACS buffer (2% FBS in 1x PBS). Spleen and Lung samples were stained with CD8-APC/Fire750 (Clone 53-6.7, BioLegend, California), CD4-PerCP-Cy5.5 (Clone GK1.5, BioLegend), B220-FITC (Clone RA3-6B2, BioLegend) at 1:200 dilution for 1hr at 4°C, fixed and permeabilized using Cytofix/Cytoperm kit (BD Biosciences). Samples were then stained with TNFα-BV421(Clone MP6-XT22, BioLegend), IFNg-PE (Clone XMG1.2, BioLegend), IL-2-APC (Clone JES6-5H4, BioLegend), diluted 1:400 for 18hr at 4°C. Samples were gated on CD8+, B220-, CD4+, TNFα+, IFNγ+ on LSR-II (Beckton Dickinson). ^{51}Chromium assay has been reported in our publications (3, 7).

Platelet Studies. 0.5 - 1 ml of blood was collected from the retro-orbital plexus into a 1.5 ml capillary tube containing 200 µl of TBS/Heparin (20 U/ml). Blood was pooled into a 15 ml conical tube with the addition of Tyrode's buffer pH 7.4 (a 2:5 ratio) then centrifuged at 100 g for 7 minutes at room temperature. Platelet-rich plasma (PRP) was removed and stored at room temperature and used within 1 hour. Platelet concentrations were measured using an IDEXX ProCyte Dx Hematology Analyzer. Platelet aggregation was performed using a 1:1 mix of infected plasma and uninfected PRP. Platelets were stimulated with 5 µmol/I ADP. Aggregation was assessed using a Chronolog Aggregometer (Model 450) with the Aggrolink software package Version 8 (Chronolog, Haverton, PA). Aggregation was measured as a maximum percentage of change in transmission of light from baseline, using platelet-poor plasma (PPP) as a reference baseline.

Statistical Analysis. Group comparisons were analyzed by ANOVA in the Prism software package. Experiments are performed in triplicate to insure reproducibility. $P < 0.05$ was considered significant.

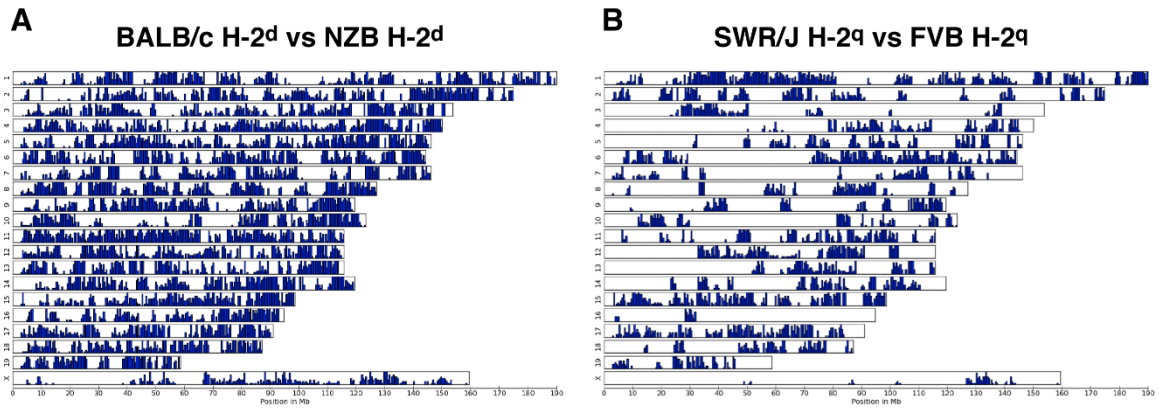

Fig. S1. Comparison of SNIP genetic mapping in H-2 strains of mice infected with Cl 13 2 x 10^6 PFU i.v. that died within 7-9 days inoculation with mice that survived infection, became persistently infected and had normal lifespans. Panel A: H-2d mice comparing BALB (survived infection, had normal lifespans and became persistently infected) with NZB (died day 7-9). Panel B: H-2q mice comparing SWR/J (survived infection, normal lifespan and became persistently infected) with FVB/N (died day 7-9).

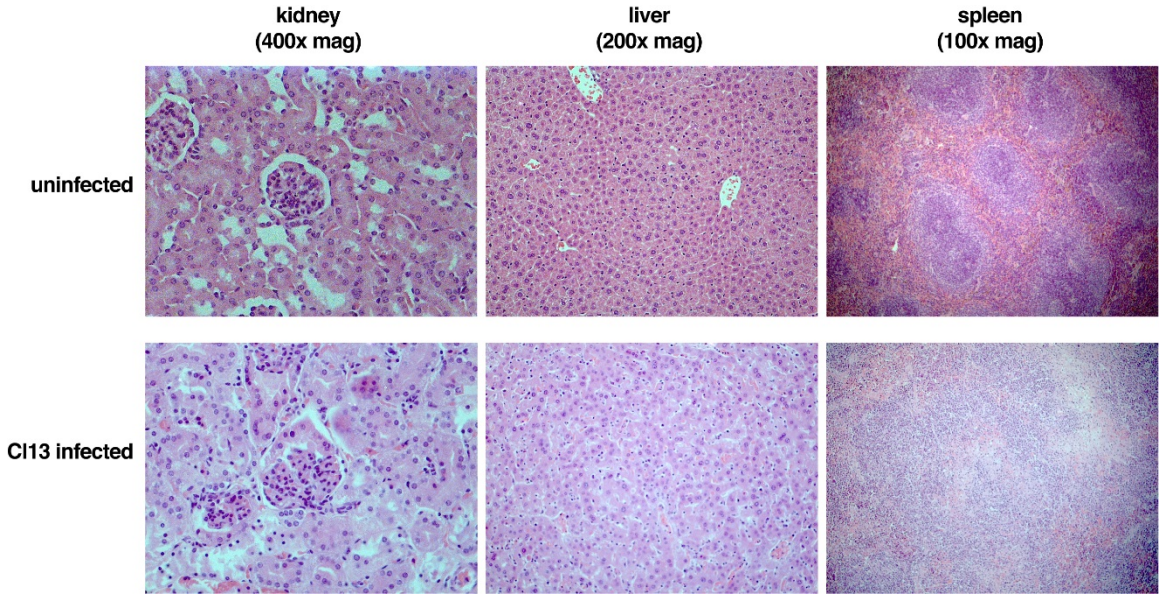

Fig. S2. Comparison of kidney, liver and spleen from NZO CC founder mice obtained 6 days after Cl 13 infection with similar tissues obtained from non-infected age- and sex-matched NZO mice.

Table S1. Panel A: death and survival of mouse strains over a 60-day observation period following 2 x 10^6 PFU of Cl 13 i.v. Lower panel displays results of Cl 13 infection on susceptible FVB/N mice x resistant C57Bl/6 mice and back-crosses of these F1 mice to FVB/N or C57Bl/6 mice. Panel B: results of ^{51}Cr lysis assay to detect CD8 T cell lysis.

A Death of mice following 2x10^6 PFU Cl13 iv

mouse strain	death 7-10 days	death >60 days
C57Bl/6	0/57[1]	2/57
Balb	0/30	3/30
SWR/J	0/15	1/15
C3H	0/4	1/4
FVB/N	16/16	–
NZB	15/15	–
PL/J	15/15	–
SJL	5/5	–
NZO	6/6	–
(C57Bl/6 x FVB/N) f1	7/7	–
(C57Bl/6 x FVB/N) f1 xC57Bl/6	4/10	6/10
(C57Bl/6 x FVB/N) f1 x FVB/N	9/10	10/10

[1]number of deaths/total number of mice

B CD8 T cell lysis at day 6 post Cl13 infection ^{51}Cr release assay

splenic CTL	E/T[2] ratio	(H-2q) NP118-126[3]	(H-2b) GP33-41[3]
FVB/N (H-2q)			
Cl13	50:1	52	4
	25:1	26	ND
ARM	50:1	35	1
	25:1	24	ND
C57Bl/6 (H-2b)			
Cl13	50:1	3	42
ARM	50:1	2	36

[2]Effector/Target ratio

[3]immunodominant epitopes for H-2q (NP118-126) and H-2b (GP33-41)

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