



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Development of transgenic mice expressing a coronavirus-specific public CD4 T cell receptor

Jingxian Zhao^{a,b,*}, Craig Fett^a, Lecia Pewe^a, Jincun Zhao^a, Stanley Perlman^a

^a Department of Microbiology, University of Iowa, Iowa City, IA, USA

^b Institute of Tissue Transplantation and Immunology, Jinan University, Guangzhou, Guangdong, China

ARTICLE INFO

Article history:

Received 13 June 2013
Received in revised form 12 July 2013
Accepted 22 July 2013
Available online 6 August 2013

Keywords:

CD4 T cell
T cell receptor
Transgenic mice
Retrogenic mice
Coronavirus

ABSTRACT

Mice that are transgenic (Tg) for T cell receptor (TCR) expression are used extensively to analyze longitudinal T cell responses during effector and memory phases of the T cell response. Generation of TCR Tg mice generally requires T cell stimulation and cloning *in vitro* prior to amplification, processes which introduce biases into selection of the TCR that is ultimately chosen for TCR Tg mouse generation. Here we describe an alternative approach that involves no T cell stimulation or propagation *in vitro*. We generated mice that were transgenic for a TCR responding to a CD4 T cell epitope (epitope M133) that is immunodominant in mice infected with a neurotropic coronavirus, the JHM strain of mouse hepatitis virus. The CD4 T cell response to epitope M133 is of particular interest because it may be pathogenic, protective or regulatory, depending upon the physiological setting. We applied an iterative process in which we identified a TCR- β chain expressed by all mice that were examined ('public sequence'). This TCR- β chain was introduced into bone marrow cells with a lentivirus vector, generating TCR- β retrogenic mice. A TCR- α chain that paired with this TCR- β was then identified and used to generate a second set of TCR (α/β) retrogenic mice. After demonstrating that these cells were functional and responded to epitope M133, these TCR chains were used to generate an epitope M133-specific TCR Tg mouse. This method should be generally useful for engineering TCR Tg mice without introduction of bias caused by *in vitro* manipulation and propagation.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

The T cell response is critical for pathogen clearance and tumor eradication and is mediated by interactions between the T cell receptor and MHC class I or II/peptide (MHCp) complex. Multiple factors, including the kinetics and affinity of the interaction, size of the T cell precursor pool, kinetics of peptide presentation and affinity of the peptide for the MHC antigen are critical for understanding the T cell response in a given physiological or pathological setting. Further, the response to a single epitope is generally complex, involving

T cells with a variety of affinities for the MHCp complex. In addition, expansion of a specific T cell in a single immune response is partly stochastic, depending on access of the T cell in question to the MHCp. Finally, the number of epitope-specific cells in the naïve T cell pool is small, ranging from less than 100 to 1000 cells (Pewe et al., 2004; Moon et al., 2007; Obar et al., 2008; La Gruta et al., 2010; Zhao et al., 2011), making it difficult to monitor endogenous T cell responses at early times after stimulation.

Many of these problems are circumvented by the use of T cell receptor (TCR) transgenic (Tg) mice. While in some cases only the TCR- β or TCR- α chain is engineered (e.g., (Turner et al., 1996), most commonly, both the alpha and beta chains of the TCR are fixed in TCR Tg mice, increasing the fraction of cells in the naïve pool that recognize the epitope in question (Barnden et al., 1998). The majority of the T cells in these mice express the Tg T cell receptor, making them an excellent

Abbreviations: CNS, central nervous system; p.i, post infection; tg, transgenic.

* Corresponding author at: Department of Microbiology, 51 Newton Road, BSB 3-712, University of Iowa, Iowa City, IA 52242, USA. Tel.: +1 319 335 8549.

E-mail address: jingxian-zhao@uiowa.edu (J. Zhao).

source for large numbers of clonotype-specific cells. Transgenic TCR T cells generally recognize MHCp complexes and function similarly to polyclonal T cells recognizing the same epitope. Development of transgenic mice is most often preceded by the generation of T cell clones specific for the epitope under study. Epitope-specific T cell clones are isolated after repeated stimulation *in vitro* and limiting dilution; it is well established that the conditions under which T cells are expanded contribute to the selection of T cells with varying affinity for the MHCp. Thus, CD8 T cells selected in the presence of low levels of antigen exhibit high affinity for MHCp whereas exposure to high levels of antigen results in the outgrowth of cells with low affinity (Alexander-Miller et al., 1996). T cells with high versus low affinity for MHCp will behave differently in an infected or cancerous animal and neither type of cell may represent a cell with average functionality in the general population of T cells responding to an epitope.

Mice infected with the neurotropic JHM strain of mouse hepatitis virus (JHMV) develop acute and chronic encephalitis and demyelination (Stohlman et al., 1998). We and others showed previously that demyelination is T cell mediated, involving virus-specific CD4 and CD8 T cells (Wang et al., 1990; Wu et al., 2000). Further, clinical disease in mice with acute encephalitis is largely CD4 T cell-driven (Anghelina et al., 2006). Thus, mutation of the immunodominant CD4 T cell epitope (M133, spanning residues 133–147 of the transmembrane M protein, TVYVRPIIEDYHTLLT) recognized in C57Bl/6 mice resulted in a loss of virulence in this strain, but not BALB/c mice (H-2^d) or in RAG1^{-/-} mice, which lack T or B cells. Virulence could be restored if another immunodominant CD4 T cell epitope (from *Listeria monocytogenes*) was introduced into the M133-mutated virus, indicating that the CD4 T cell response was pathogenic. On the other hand, our results also demonstrated that the memory CD4 T cell response was protective. In another report, we showed that a population of regulatory CD4 T cells in the brains of mice infected with a neuroattenuated variant of JHMV (rJ2.2) recognized epitope M133 (Zhao et al., 2011). Understanding the relationship between effector, memory and regulatory CD4 T cells responding to a single epitope would be facilitated by the availability of an M133-specific TCR Tg mouse.

Here, we describe an approach to developing TCR Tg mice that does not require *in vitro* cell culturing or limiting dilution. The beta chain of an M133-specific CD4 T cell receptor was initially selected on the basis of its expression in 3/3 JHMV-infected mice. The alpha chain was then selected using infected single chain TCR retrogenic mice in which the beta chain identified in the initial studies was fixed. Subsequent analyses showed that these M133-specific TCR Tg cells, upon transfer to mice prior to infection with rJ2.2, proliferated and trafficked to the infected brain.

2. Materials and methods

2.1. Mice

Specific pathogen-free 6 week old C57BL/6 (B6) and Thy1.1 congenic mice were purchased from the National Cancer Institute. Mice were maintained in the animal care facility at the University of Iowa. After viral inoculation, mice were examined and weighed daily. Clinical evaluation was based on

the following scoring system: 0, asymptomatic; 1, limp tail or slightly hunched; 2, wobbly gait or hunched and ruffled fur; 3, hindlimb paresis or moderate wasting; 4, hindlimb paralysis or severe wasting; 5, moribund. All animal studies were approved by the University of Iowa Animal Care and Use Committee (Iowa City, IA).

2.2. Virus

Neurovirulent JHMV and its neuroattenuated variant, rJ2.2 (a recombinant version of the J2.2-V-1 virus) were grown and titered as described (Pewe et al., 2005). B6 mice were infected intraperitoneally with 1.5×10^5 PFU JHMV or intracranially with 600–700 PFU of rJ2.2.

2.3. IFN- γ capture assay

Cells were harvested from the spleens of JHMV-infected B6 mice 7 days after infection. To isolate epitope M133-specific CD4 T cells, 1×10^7 unfractionated splenocytes/ml were stimulated 1:2 with CHB3 cells for 4 h with $1 \mu\text{M}$ of M133 peptide (TVYVRPIIEDYHTLLT) (Xue et al., 1995). After stimulation, M133-specific CD4 T cells were identified using a mouse IFN- γ secretion assay kit (Miltenyi Biotec, Auburn, CA) following the manufacturer's protocol for frequencies of IFN- γ -secreting T cells <2%. IFN- γ ⁺ cells were detected with PE-conjugated anti-IFN- γ antibody. Cells were additionally stained with FITC-conjugated anti-CD4 Abs. IFN- γ -expressing CD4 T cells were sorted with a FACS Diva (BD Biosciences, San Jose, CA). A total of 40,000–50,000 epitope M133-specific cells were obtained from each sort. Under our sorting conditions, very few IFN- γ -expressing CD4 T cells were detected when cells were stimulated in the absence of added peptide (Fig. 1).

2.4. Isolation of RNA from lymphocytes and TCR- β sequence analysis

RNA was isolated from lymphocytes using an RNeasy Mini Kit (Qiagen, Valencia, CA). cDNA was synthesized from each entire sorted lymphocyte RNA preparation in a 60 μl reaction, as described (Pewe et al., 1996). A total of 5 μl of cDNA was used for PCR. V β 8 was the commonly used V β element in the M133-specific CD4 T cell response (Haring et al., 2001). For synthesis of V β 8-specific PCR products, a V β 8-specific forward primer (5'-CATGGGCTGAGCTGATCCATT-3') was used with a common C β reverse primer (5'-GCAATCTCTGCTTTTGATGGCTC-3') and Taq polymerase (AmpliTaQ Gold DNA Polymerase; Applied Biosystems, Foster City, CA). Amplification was conducted for 25 cycles, as described previously (Pewe et al., 1996). The PCR error rate was 0.12% (Pewe et al., 2004). PCR products were cloned into plasmid vector pCR2.1-TOPO, as described by the manufacturer (Invitrogen, Carlsbad, CA). Colonies containing inserts were identified and sequenced with an automated ABI Prism 3700 Sequencer (Applied Biosystems).

2.5. Antibodies

The following antibodies were used in this study: CD4-PerCP-Cy5.5 or -FITC (RM 4–5), TCRV β 8.3-FITC or -PE (1B3.3) (BD Pharmingen, San Diego, CA); CD8-APC (53-6.7), CD16/CD32-biotin (93), IFN- γ -allophycocyanin (APC; XMG1.2),

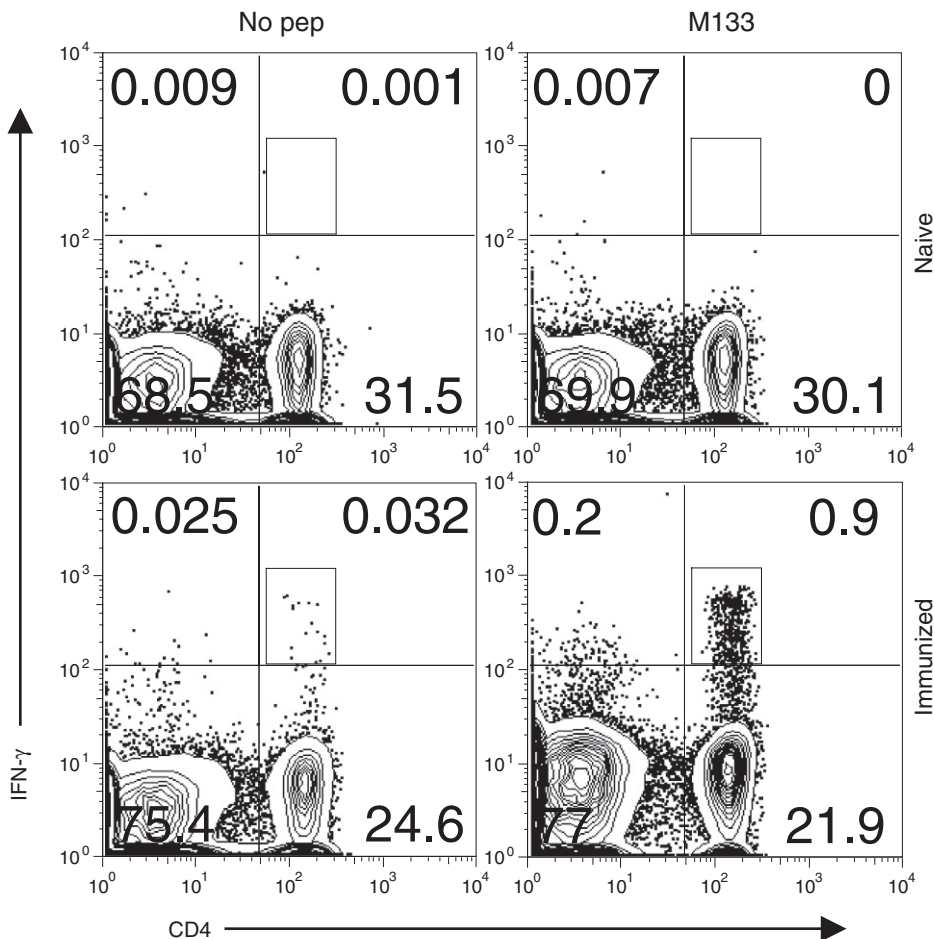


Fig. 1. Specificity of M133-specific T cell sorting after staining with IFN- γ capture assay. Cells were harvested from the spleen of a naive B6 mouse or a mouse infected i.p. with JHMV and stained for IFN- γ production in response to peptide M133 or no peptide using an IFN- γ capture assay. Gates for sorting were chosen to minimize nonspecific staining.

TNF-PE (MP6-XT22), TCR α 2-PE or -APC (B20.1) (eBioscience, San Diego, CA); IL-10-APC (JES5-16E3) (BioLegend, San Diego, CA).

2.6. MHC class II/peptide M133 tetramer staining

Heparinized whole blood or central nervous system (CNS)-derived mononuclear cells were stained with 8 μ g/ml PE-conjugated I-A^b/M133 tetramers (obtained from the National Institutes of Health/National Institute of Allergy and Infectious Diseases MHC Tetramer Core Facility, Atlanta, GA) in RP10 media for 1 h at 37 °C. Cells were then incubated with anti-CD16/CD32-biotin and anti-CD4-PerCP-Cy5.5, followed by streptavidin-APC and were analyzed using a FACSCalibur (BD Biosciences). Results are shown after gating on CD16/CD32-negative cell populations.

2.7. Isolation of mononuclear cells from brains

Brains harvested after PBS perfusion were dispersed and digested with 1 mg/ml collagenase D (Roche Applied Science, Indianapolis, IN) and 0.1 mg/ml DNase I (Roche Applied

Science) at 37 °C for 30 min. Dissociated brain tissue was passed through a 70- μ m cell strainer, followed by Percoll gradient (70/37%) centrifugation. Mononuclear cells were collected from the interphase, washed, and resuspended in culture medium for further analysis.

2.8. Development of M133-specific TCR- β retrogenic mice

M133 TCR- β retrogenic mice were engineered as previously described (Holst et al., 2006a; Holst et al., 2006b; Zhao et al., 2011). Briefly, an M133-specific TCR- β (V β 8.3, TRBV13-1) was produced synthetically (GeneArt, Invitrogen, Regensburg, Germany) and cloned into a retroviral vector containing an IRES-GFP cassette to allow identification of transfected cells by GFP expression (MSCV-IRES-GFP, pMIG, kindly provided by Dr. Dario Vignali, St. Jude Children's Hospital). Retroviral producer cells were sorted based on GFP expression and expanded. These cells produced retroviral particles that were coated with the VSV glycoprotein (G) and expressed M133 TCR- β . B6 donor bone marrow cells were transduced with these particles and then infused into lethally irradiated (950 rad) recipient B6 mice as described previously (Holst et al., 2006a;

Holst et al., 2006b). Mice were infected with rJ2.2 at 6–10 weeks after reconstitution. The majority of CD4 T cells in the infected CNS were GFP⁺. Using I-A^b/M133 tetramers (NIH Tetramer Facility, Atlanta, GA), we sorted 7.5×10^5 M133-specific GFP⁺ cells from the infected CNS and analyzed them for TCR- α chain expression. For this purpose, a panel of pooled V α primers (pool 1: V α 1: GCACTGATGTCATCTTCCTC; V α 2: AAAGGGAGAAAAAGCTCTCC; V α 3: AAGTACTATCCGGAGACCC; V α 4: CAGTATCCCGGAGAAGGTC; pool 2: V α 5: CAAGAAAAGACA AACGACTCTC; V α 6: ATGGCTTTCTGGCTATTGCC; V α 7: TCTGTAGTCTCCAGAAATC; V α 8: CAACAAGAGGACCGAGCACC; pool 3: V α 9: TAGTGACTGTGGTGGATGTC; V α 10: AACGTCCGAGCTCTTTGCAC; V α 11: CCCTGCACATCAGGGATGCC; V α 12: TCTGTTTATCTCTGCTGACC; pool 4: V α 13: ACCTGGAGAGAA TCCTAAGC; V α 14: TCCTGGTTGACCAAAAAGAC; V α 16: CATT CGCTCAAATGTGAACAG; V α 17: GGAAAATGCAACAGTGGGTC; pool 5: V α 18: CAAATGAGAGAGAGAAGCGC; V α 19: TGGTTT GAAGGACAGTGGGC; V α 20: GACATGACTGGCTTCTGAAAGCC TTGC) was used in conjunction with a common C α reverse primer (TGGCGTTGGTCTCTTTGAAG). Four TCR- α chains were identified, differing only by single amino acid in the CDR3 region. One of these TCR- α chains was produced synthetically and cloned into the retroviral vector described above (Holst et al., 2006a). In this vector, the TCR- α and TCR- β chains are linked via a self-cleaving protease (protease 2A), enabling stoichiometric production of the two chains.

2.9. Development of M133-specific TCR transgenic (Tg) mice

M133 TCR Tg mice were engineered as previously described (Barnden et al., 1998). Briefly, the TCR- α and TCR- β chains identified above were cloned into plasmids pES4 and p3A9c β TCR, respectively (kindly provided by Dr. Frank Carbone, University of Melbourne). The transgenes were excised, purified, and microinjected into pronuclei of B6SJL mice (C57Bl/6 J \times SJL/J F2). Genotyping was performed by PCR of tail DNA using the following primers: TCR- α (forward) 5'- CCCTTGGTATCAGCAGTTC-3' and (reverse) 5'- GGT AGGTGGCGTTTGTCTCT-3'; TCR- β (forward) 5'-TGACATC ATCAGGCTTTGTCTT-3' and (reverse) 5'- CCCAGCTCACCTAACA CGAG-3'. Mice were backcrossed onto a B6 mouse background using speed congenic genotyping (Harlan Laboratories). After 4 rounds of backcrossing, mice were 99.7% identical to B6 mice, as measured by SNP analysis. Mice were maintained by backcrossing with B6 mice. After four additional generations, male and female TCR Tg mice were crossed to develop mice that were homozygous for the transgene.

2.10. Intracellular cytokine staining

To detect cytokine production by antigen-specific CD4 T cells, mononuclear cells isolated from the brain were stimulated with 5 μ M of peptide M133 in complete RPMI 1640 medium for 6 h at 37 °C, in the presence of 1 μ l/ml Golgiplug (BD Pharmingen) and antigen-presenting cells (CHB3 cells, B cell line, H-2D^b, I-A^b). Intracellular expression of IFN- γ , IL-10, and TNF was assayed.

2.11. Adoptive transfer

M133 TCR Tg mice were crossed onto a Thy1.1 background. Splenocytes were harvested from these mice and

CD4 T cells enriched using magnetic beads (Miltenyi). 10⁵ cells were transferred to 6 week B6 mice 24 h prior to rJ2.2 infection.

2.12. Statistics

Two-tailed, unpaired Student *t* tests were used to analyze differences in mean values between groups. All results are expressed as means \pm SEM. *P* values < 0.05 were considered significant.

3. Results

3.1. Identification of the β chains of M133-specific T cell receptors

In previous studies designed to determine the diversity of the T cell response to single epitopes, we developed methods to isolate virus epitope-specific T cells and prepare cDNA clones encompassing the CDR3 region (Pewe et al., 1999; Pewe et al., 2004). These studies described diversity of the T cell response to CD8 T cell epitopes recognized in B6 mice infected with JHMV (epitopes S510 and S598) or lymphocytic choriomeningitis virus (LCMV, epitope gp33). We next extended our analyses to the immunodominant CD4 T cell epitope (epitope M133) recognized in B6 mice (Xue et al., 1995). Up to 25% of the CD4 T cells in the acutely infected brain respond to epitope M133 (Zhao et al., 2009). At the time that these experiments were initiated, an I-A^b/M133 tetramer was not available so we identified epitope-specific cells using an IFN- γ capture assay, as described in Materials and Methods. The IFN- γ capture assay captured no more than 50% of M133-specific T cells (data not shown), making analyses of infected brains difficult. Therefore, in order to obtain sufficient cells for analysis, we stained and sorted M133-specific cells from the spleens of mice after intraperitoneal infection with JHMV. As shown in Fig. 1, M133-specific CD4 T cells were readily detected in the spleen using an IFN- γ capture assay. Next, these CD4 T cells were sorted from three individual mice and RNA prepared. V β 8 is the most commonly expressed V β element, used by approximately 20% of all M133-specific CD4 T cells (Haring et al., 2001), so primers encoding V β 8 and C β sequences were used to develop cDNA clones that spanned the CDR3. The CDR3 is the region of greatest variability in TCR- α and TCR- β chains so can be used to determine the diversity of the response. 87–100 cDNA clones were sequenced from each mouse. The results indicated that there was extensive TCR- β chain diversity, with 39–46 unique β chains identified per mouse (Table 1).

3.2. Generation of TCR retrogenic mice

To begin the process of engineering a TCR Tg mouse, we identified several CDR3 sequences that were common to all three mice (Table 2). One TCR- β chain, containing TRBV13-1 and TRBJ2-2 elements (CDR3: ARDM) was abundant in 2/3 mice and was chosen for further studies. A complete TCR- β chain was produced and single chain M133 TCR retrogenic mice were developed, as described in Materials and Methods. By 5 weeks after reconstitution, 20–35% of CD4 T cells in the blood of each mouse expressed GFP (Fig. 2A). After infection

with rJ2.2, these cells trafficked to the brain, comprising 55% of the total and >90% of the M133-specific CD4 T cell response (Fig. 2B). To identify the TCR- α chain(s) associated with the fixed TCR- β chain, cells were stained with I-A^b/M133 tetramers and tetramer⁺GFP⁺ CD4 T cells were sorted. RNA was isolated from these cells and used to prepare cDNA. Using a panel of pools of PCR primers for the most commonly used V α elements, we sequenced 71 cDNA clones. Twenty four of these 71 cDNA clones contained in frame sequences and all 24 cDNA clones encoded the V α 2.3 (TRAV14-1) and J α 33 (TRAJ33) elements, with the following sequences: **CASS [DP (11 clones)/AP (11 clones)/LP (1 clone)/AG (1 clone)] TGNKYVFGA**. Additional sequence analysis revealed that the α chain contained the TRAV14-1*01 element. A complete M133 TCR- α containing one of the common CDR3 (AP) was expressed synthetically, and introduced into the same retroviral transduction vector as above. Retrogenic mice expressing both the M133 TCR- α and TCR- β chains were generated by bone marrow transduction and reconstitution of lethally irradiated mice (Fig. 3A). CD4 T cells expressing the retrogenic M133-specific TCR trafficked to the CNS after infection with rJ2.2 and expressed both IFN- γ and IL-10 after peptide stimulation directly *ex vivo* (Fig. 3B, C).

3.3. Generation and rJ2.2 infection of M133 TCR Tg mice

While M133 TCR retrogenic mice are useful for many studies, M133-specific CD4 T cells comprised only a minority of the total CD4 T cell population in naïve mice and there was sufficient variability in numbers of M133-specific cells between different M133 TCR retrogenic mice to make direct comparisons after infection difficult. Therefore, to develop a reliable supply of M133-specific cells, we engineered a transgenic mouse expressing the same TCR as used in the TCR retrogenic mice as described in Materials and Methods (section 2.9). Unlike the TCR retrogenic mice, M133-specific cells in the Tg mice did not express GFP, limiting detection to use of I-A^b/M133 tetramers or dual V β 8.3/V α 2 staining. As expected for a TCR Tg mouse recognizing a CD4 T cell epitope, the great majority of T cells in the blood were CD4⁺ (Fig. 4A). Further, M133-specific CD4 T cells were readily detected in the blood of the TCR transgenic mice, after staining with either anti-V β 8.3/V α 2 antibodies (Fig. 4B) or I-A^b/M133 tetramers (Fig. 4C). Homozygous Tg mice bred normally and no physiological abnormalities were observed.

We next assessed the functionality of these M133 TCR Tg CD4 T cells. First, we directly infected M133 TCR Tg mice with rJ2.2. Infection of wild type B6 mice with 700 PFU of rJ2.2 results in mild encephalitis, with a mortality of 0–20% during the first 10 days after infection (Zhao et al., 2009). A variable percentage then develops hindlimb paralysis and

Table 1

Diversity of epitope M133-specific CD4 T cells in spleen.

Mouse	No. of cDNA clones analyzed	No. of different cDNA clones
1	87	39
2	90	46
3	100	40

Table 2

M133-specific TCR- β chain CDR3 common to all mice analyzed.

Clone no.	V	CDR3	J	Jb	Frequency/mouse ^a		
					1	2	3
1	ASS	ARDMNTGQL ^b	YFG	2.2	8/87	5/90	1/100
2	ASG	GGGTGLNLT	YFG	2.4	1/87	4/90	2/100
3	ASS	GTVNLT	YFG	2.4	1/87	2/90	3/100
4	ASS	DWGDQDTQ	YFG	2.5	10/87	1/90	5/100
5	ASR	DWGSQDTQ	YFG	2.5	2/87	3/90	1/100
6	ASG	RDWGGYEQ	YFG	2.6	1/87	2/90	2/100

^a Clones/total number cDNA clones sequenced in each mouse.

^b This TCR- β chain was used to develop M133 TCR retrogenic and M133 TCR Tg mice.

demyelination on histological examination over the following 1–2 weeks. In marked contrast, infected M133 TCR Tg mice developed severe clinical disease, succumbing to the infection by day 13–15 post infection (p.i.) (Fig. 4D). Notably, infected M133 TCR Tg mice exhibited diminished weight loss compared to B6 mice at early times p.i., even though the great majority eventually succumbed to the infection.

Previously, we showed that M133-specific CD4 T cells were pathogenic in mice acutely infected with neurovirulent JHMV (Anghelina et al., 2006) but the role of these cells in rJ2.2-infected mice was not investigated in those studies; the results in Fig. 4 suggest that M133-specific CD4 T cells were pathogenic even in a neuroattenuated infection if the CD4 T cell response was primarily directed at this epitope. To verify that the T cell response at the site of inflammation was epitope M133-specific, we harvested lymphocytes from the brains of infected M133 TCR Tg mice and analyzed them directly *ex vivo* for cytokine expression. At both days 7 and 14 p.i., the majority of cells in the infected brain predominantly responded to epitope M133 (Fig. 4E). Unexpectedly, the majority of cells at day 7 p.i. expressed TNF but not IFN- γ but by 14 days p.i., the predominant populations expressed IFN- γ . Whether this temporal difference in cytokine expression contributes to disease pathogenesis requires further investigation. These M133-specific CD4 T cells were not able, however, to clear virus from the infected brain (Fig. 4F). Virus is cleared by 10–14 days p.i. from the brains of B6 mice ((Parra et al., 1999), Fig. 4F). However, in marked contrast, virus titers in M133 TCR Tg mice were elevated compared to B6 mice and did not diminish between days 7 and 10 p.i., providing an explanation for the increased mortality observed in the M133 TCR Tg mice. These stably high virus titers reflect a requirement for an effective anti-virus CD8 T cell response for optimal virus clearance (Bergmann et al., 2006) and a lack of MHC class II expression on oligodendrocytes, the predominant cell type infected in the brain (Redwine et al., 2001).

3.4. Use of M133-specific TCR Tg CD4 T cells for tracking studies

To prove the utility of these mice in tracking studies, we crossed the M133 TCR Tg mice onto a Thy1.1 background and transferred 10⁵ CD4 T cells to 6 week old B6 mice 24 h prior to infection with rJ2.2. At day one p.i., these cells could be

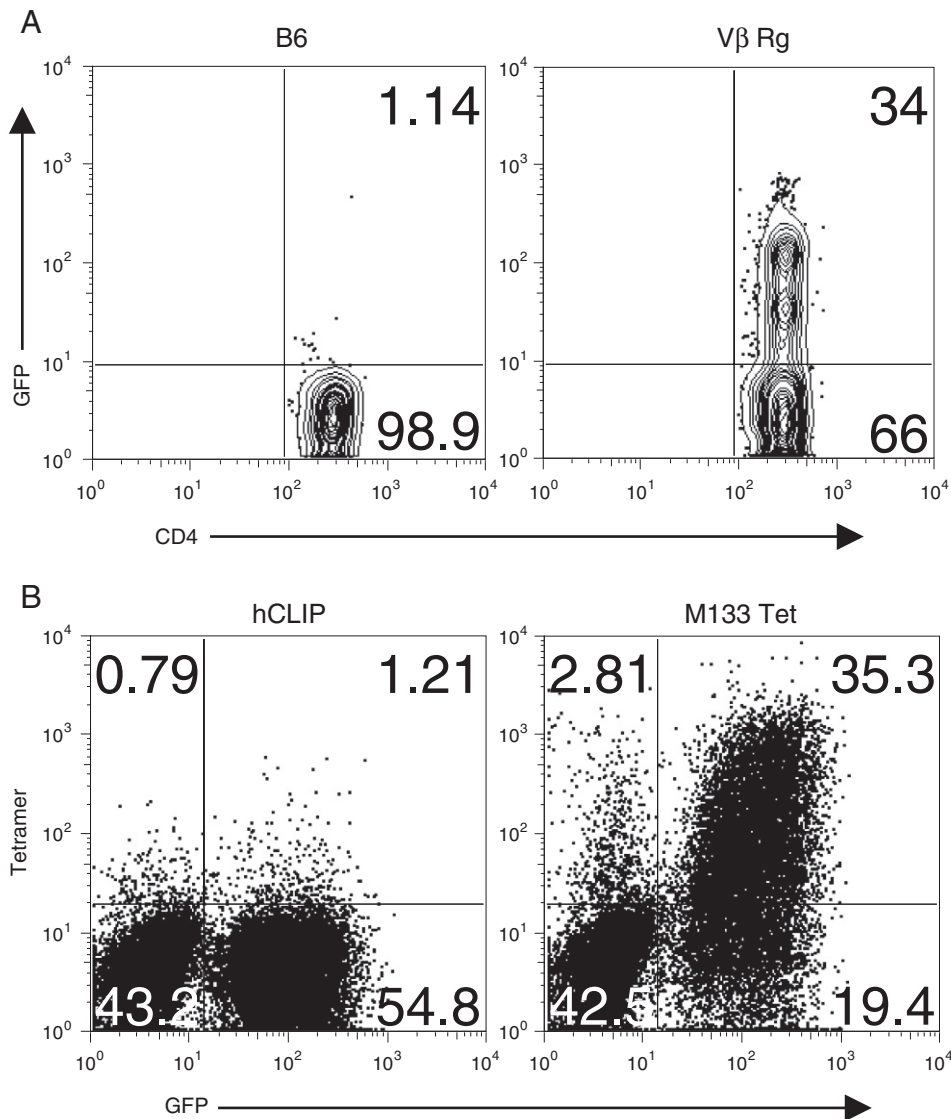


Fig. 2. Analysis of CD4 T cells from the blood and brains of TCR- β retrogenic mice. A. Blood was obtained from M133 TCR- β retrogenic mice at 6 weeks after bone marrow infusion and analyzed for the expression of GFP. Cells from B6 mice were used as a control. B. TCR- β retrogenic mice were infected with rJ2.2. Mice were sacrificed at d. 7 p.i. and brain-derived lymphocytes were prepared and stained with I-A^b/M133 or I-A^b/hCLIP (control) tetramers. Cells were gated for CD4 expression.

detected in the spleen and lymph nodes at very low levels (Fig. 5A). By day 8 p.i., these cells proliferated and migrated to the brain, accounting for $51 \pm 6.6\%$ ($n = 4$) of the total CD4 T cell response (Fig. 5B).

4. Discussion

In the current study, we describe a modified method for generating TCR Tg mice that does not depend upon repeated antigen stimulation or limiting dilution *in vitro*. The resultant mouse encodes a TCR selected on the basis of its “public” sequence based on its detection in all of the mice examined. In this approach, the TCR- β chain was identified initially and on further analysis, using TCR- β retrogenic mice, was found to pair with a limited number of closely related TCR- α chains,

simplifying selection of the second TCR chain. Thus, consistent with previous reports, fixation of one of the TCR chains resulted in very limited diversity in the other chain, illustrating how only some combinations of TCR- α and TCR- β chains allow recognition of specific peptide/MHC complexes (Turner et al., 1996). After adoptive transfer, M133 TCR Tg cells are useful for tracking the epitope-specific CD4 T cell response. This will be useful in mice infected with neurotropic strains of murine coronaviruses, in which cells responding to the M133 epitope are pathogenic (primary), protective (memory) or immunomodulatory (Treg) (Anghelina et al., 2006; Savarin et al., 2008; Zhao et al., 2011).

In summary, this approach should be generally useful for generating related sets of epitope-specific TCR retrogenic or transgenic mice expressing public sequences, since it is

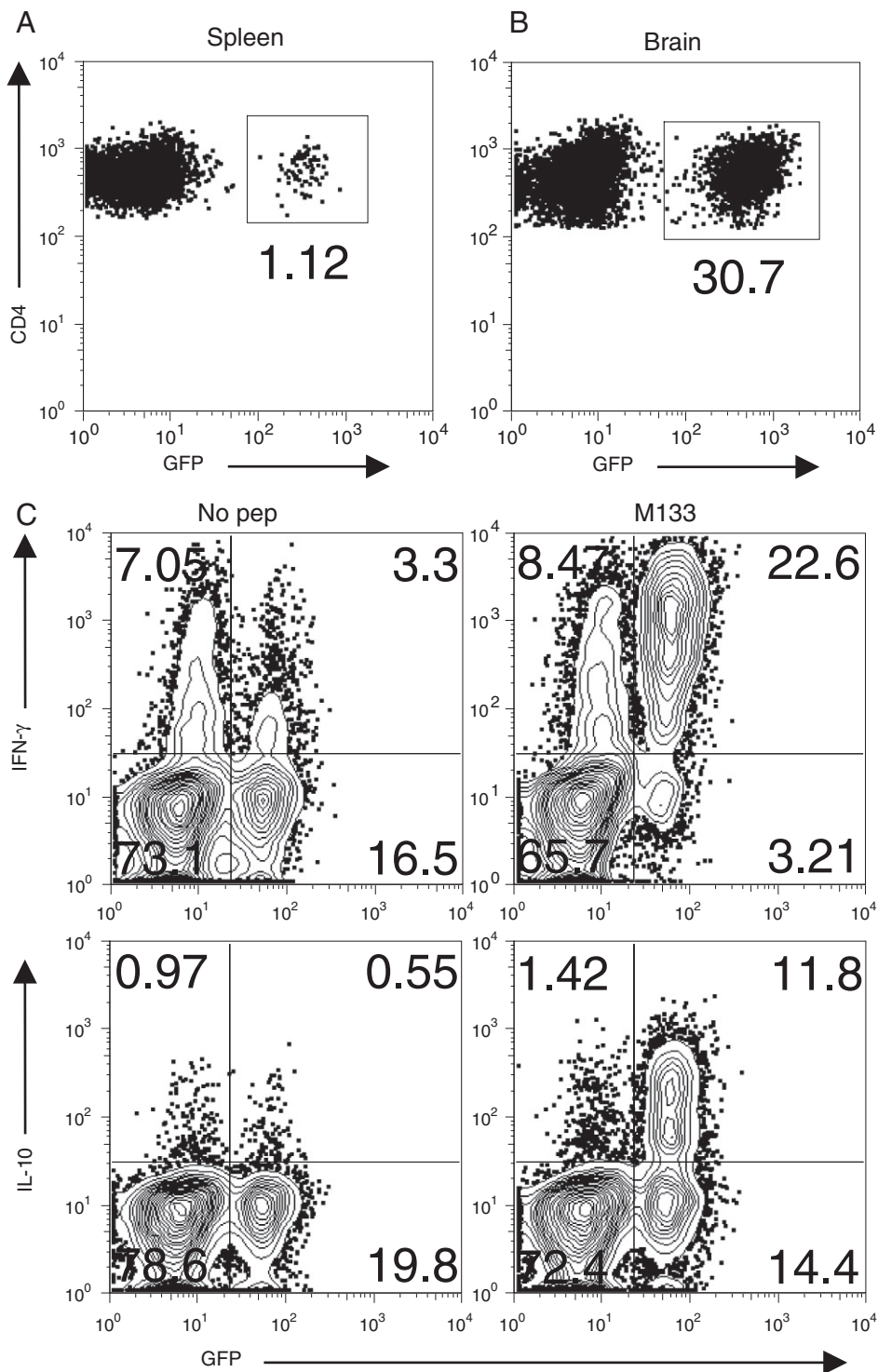


Fig. 3. Analysis of CD4 T cells from the spleens and brains of M133 TCR- α/β retrogenic mice. (A) Spleens were harvested from uninfected TCR- α/β retrogenic mice and analyzed for the presence of M133-specific (GFP⁺) CD4 T cells. (B,C) TCR- α/β retrogenic mice were infected with rJ2.2 and lymphocytes were prepared from infected brains. Cells were gated for CD4 expression and then analyzed for GFP expression (B) and the expression of IFN- γ and IL-10 after M133 peptide stimulation (C). Approximately 90% and 45% of GFP⁺ cells expressed IFN- γ and IL-10, respectively.

simple to determine TCR- β chain sequences once epitope-specific cells have been selected by MHC/peptide tetramer staining or an IFN- γ capture assay. Alternatively, TCRs that are

public can be identified using single cell RT-PCR techniques (Dash et al., 2011). These mice will allow for probing of the relationship between T cell clonotypes that are present in the

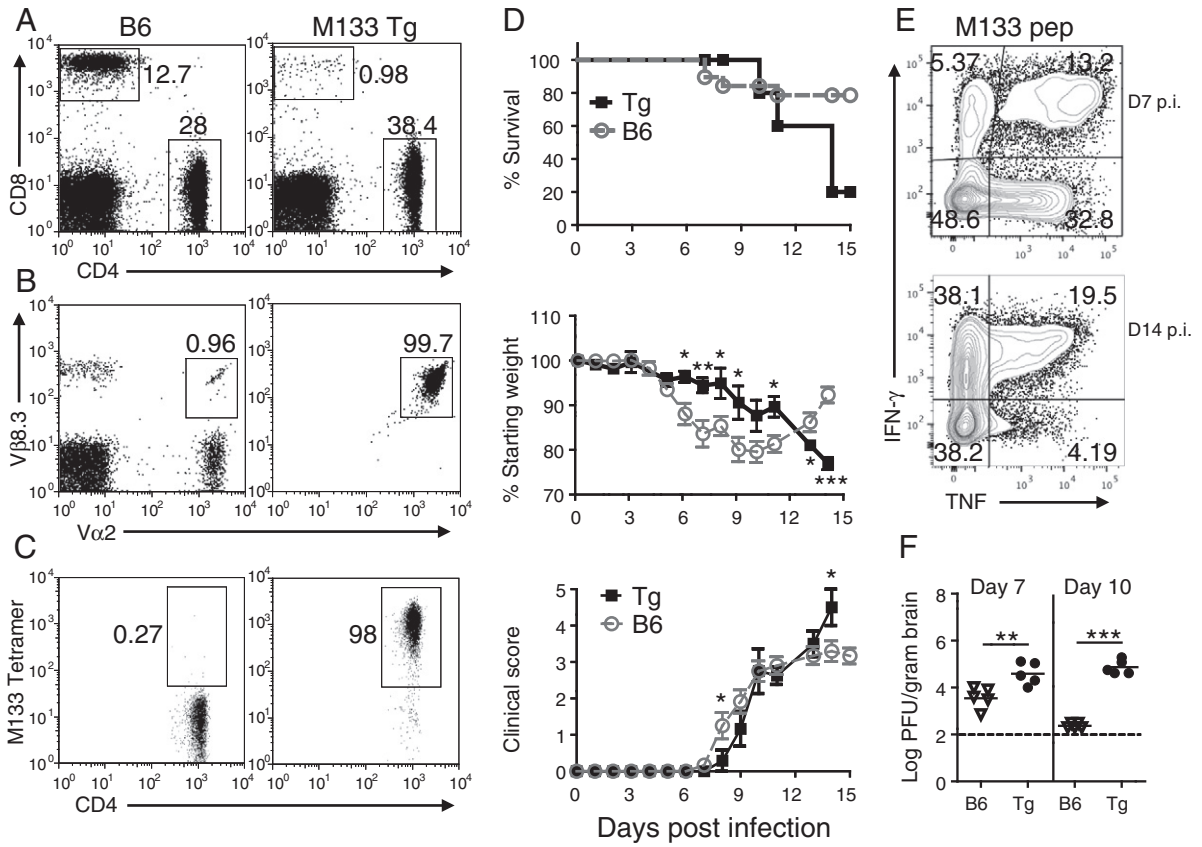


Fig. 4. rJ2.2 infection of naïve and M133 TCR Tg mice. (A–C) Blood was obtained from uninfected M133 TCR Tg mice and analyzed for (A) CD4 and CD8 T cell populations and (B) Vα2 and Vβ8.3 expression and (C) tetramer I-A^b/M133 binding by CD4 T cells. (D) M133 TCR Tg mice were infected i.c. with rJ2.2 and analyzed for survival, weight loss and clinical disease ($n = 10$ B6 and 8 Tg mice). (E) Cells were harvested from the CNS of Tg mice at days 7 and 14 post infection. CD4 T cells were analyzed for cytokine expression after M133 peptide stimulation. (F) Virus titers were measured in the brains of B6 and M133 TCR Tg mice at days 7 and 10 p.i. Titers for individual mice are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Dashed line represents limit of detection.

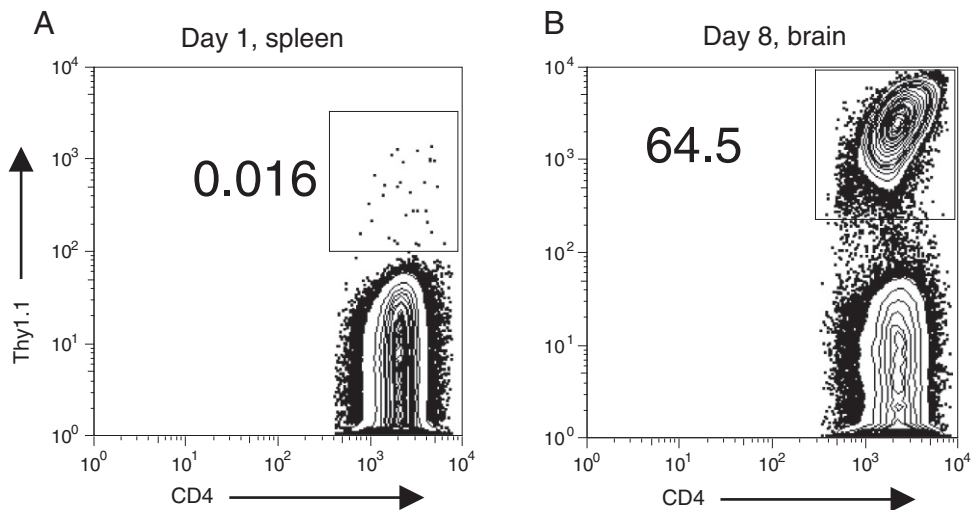


Fig. 5. rJ2.2-infection of mice after adoptive transfer of M133 TCR Tg CD4 T cells. CD4 T cells were enriched from M133 TCR Tg Thy1.1 mice and 10^5 cells were transferred to B6 mice 24 h prior to rJ2.2 infection. Cell numbers in the spleen at day 1 and brain at day 8 p.i. are shown.

precursor pool in all mice (those bearing public sequences) and functional avidity and, in the context of infection, protective function.

Acknowledgments

We thank the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases Tetramer Core Facility (Atlanta, GA) for providing tetramers and Dr. D. Vignali (St. Jude's Research Hospital) and Drs. F. Carbone and C. Jones (University of Melbourne, Melbourne, AU) for reagents and guidance as we developed M133-specific TCR retrogenic and TCR Tg mice, respectively. We also thank the University of Iowa Flow Cytometry facility for help with cell sorting. This work was supported in part by grants from the NIH (NS36592) and National Multiple Sclerosis Society (RG 2864-E-6). The authors declare no competing financial interests.

References

- Alexander-Miller, M.A., Leggatt, G.R., Berzofsky, J.A., 1996. Selective expansion of high- or low-avidity cytotoxic T lymphocytes and efficacy for adoptive immunotherapy. *Proc. Natl. Acad. Sci.* 93, 4102.
- Anghelina, D., Pewe, L., Perlman, S., 2006. Pathogenic role for virus-specific CD4 T cells in mice with coronavirus-induced acute encephalitis. *Am. J. Pathol.* 169, 209.
- Barnden, M.J., Allison, J., Heath, W.R., Carbone, F.R., 1998. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* 76, 34.
- Bergmann, C.C., Lane, T.E., Stohlman, S.A., 2006. Coronavirus infection of the central nervous system: host-virus stand-off. *Nat. Rev. Microbiol.* 4, 121.
- Dash, P., McClaren, J.L., Oguin III, T.H., Rothwell, W., Todd, B., Morris, M.Y., Becksfort, J., Reynolds, C., Brown, S.A., Doherty, P.C., Thomas, P.G., 2011. Paired analysis of TCRalpha and TCRbeta chains at the single-cell level in mice. *J. Clin. Invest.* 121, 288.
- Haring, J.S., Pewe, L.L., Perlman, S., 2001. High-magnitude, virus-specific CD4 T-cell response in the central nervous system of coronavirus-infected mice. *J. Virol.* 75, 3043.
- Holst, J., Szymczak-Workman, A.L., Vignali, K.M., Burton, A.R., Workman, C.J., Vignali, D.A., 2006a. Generation of T-cell receptor retrogenic mice. *Nat. Protoc.* 1, 406.
- Holst, J., Vignali, K.M., Burton, A.R., Vignali, D.A., 2006b. Rapid analysis of T-cell selection in vivo using T cell-receptor retrogenic mice. *Nat. Methods* 3, 191.
- La Gruta, N.L., Rothwell, W.T., Cukalac, T., Swan, N.G., Valkenburg, S.A., Kedzierska, K., Thomas, P.G., Doherty, P.C., Turner, S.J., 2010. Primary CTL response magnitude in mice is determined by the extent of naive T cell recruitment and subsequent clonal expansion. *J. Clin. Invest.* 120, 1885.
- Moon, J.J., Chu, H.H., Pepper, M., McSorley, S.J., Jameson, S.C., Kedl, R.M., Jenkins, M.K., 2007. Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 27, 203.
- Obar, J.J., Khanna, K.M., Lefrancois, L., 2008. Endogenous naive CD8 + T cell precursor frequency regulates primary and memory responses to infection. *Immunity* 28, 859.
- Parra, B., Hinton, D., Marten, N., Bergmann, C., Lin, M.T., Yang, C.S., Stohlman, S.A., 1999. IFN-gamma is required for viral clearance from central nervous system oligodendroglia. *J. Immunol.* 162, 1641.
- Pewe, L., Wu, G., Barnett, E.M., Castro, R., Perlman, S., 1996. Cytotoxic T cell-resistant variants are selected in a virus-induced demyelinating disease. *Immunity* 5, 253.
- Pewe, L., Heard, S.B., Bergmann, C.C., Dailey, M.O., Perlman, S., 1999. Selection of CTL escape mutants in mice infected with a neurotropic coronavirus: quantitative estimate of TCR diversity in the infected CNS. *J. Immunol.* 163, 6106.
- Pewe, L.L., Netland, J.M., Heard, S.B., Perlman, S., 2004. Very diverse CD8 T cell clonotypic responses after virus infections. *J. Immunol.* 172, 3151.
- Pewe, L., Zhou, H., Netland, J., Tangadu, C., Olivares, H., Shi, L., Look, D., Gallagher, T.M., Perlman, S., 2005. A severe acute respiratory syndrome-associated coronavirus-specific protein enhances virulence of an attenuated murine coronavirus. *J. Virol.* 79, 11335.
- Redwine, J.M., Buchmeier, M.J., Evans, C.F., 2001. In vivo expression of major histocompatibility complex molecules on oligodendrocytes and neurons during viral infection. *Am. J. Pathol.* 159, 1219.
- Savarin, C., Bergmann, C.C., Hinton, D.R., Ransohoff, R.M., Stohlman, S.A., 2008. Memory CD4+ T-cell-mediated protection from lethal coronavirus encephalomyelitis. *J. Virol.* 82, 12432.
- Stohlman, S.A., Bergmann, C.C., Perlman, S., 1998. Mouse hepatitis virus. In: Ahmed, R., Chen, I. (Eds.), *Persistent Viral Infections*. John Wiley & Sons, Ltd., New York, p. 537.
- Turner, S.J., Cose, S.C., Carbone, F.R., 1996. TCR alpha-chain usage can determine antigen-selected TCR beta-chain repertoire diversity. *J. Immunol.* 157, 4979.
- Wang, F., Stohlman, S.A., Fleming, J.O., 1990. Demyelination induced by murine hepatitis virus JHM strain (MHV-4) is immunologically mediated. *J. Neuroimmunol.* 30, 31.
- Wu, G.F., Dandekar, A.A., Pewe, L., Perlman, S., 2000. CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. *J. Immunol.* 165, 2278.
- Xue, S., Jaszewski, A., Perlman, S., 1995. Identification of a CD4+ T cell epitope within the M protein of a neurotropic coronavirus. *Virology* 208, 173.
- Zhao, J., Zhao, J., Perlman, S., 2009. De novo recruitment of antigen-experienced and naive T cells contributes to the long-term maintenance of antiviral T cell populations in the persistently infected central nervous system. *J. Immunol.* 183, 5163.
- Zhao, J., Zhao, J., Fett, C., Trandem, K., Fleming, E., Perlman, S., 2011. IFN-gamma- and IL-10-expressing virus epitope-specific Foxp3(+) T reg cells in the central nervous system during encephalomyelitis. *J. Exp. Med.* 208, 1571.